THE ACTION OF NEOMYCIN AND LITHIUM ON MAMMALIAN SLOWLY ADAPTING TYPE I MECHANORECEPTOR: A POSSIBLE ROLE OF PHOSPHOINOSITIDE METABOLISM

by

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A thesis submitted as partial fulfilment of the requirement for the degree of Master of Philosophy

June, 1987

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CONTENT

ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincerest gratitude to my supervisor, Professor W. Hamann for his constant supervision and stimulating discussion throughout the entire period of the study. His experimental know-how has benefited me a lot and his encouragement and patience especially during the initial period of my study are most appreciated. My very special thank goes to Dr. K. Baumann for his care and encouragement throughout the past two years, and also for his valuable advice especially on microcomputing and data processing and analysis. I am also indebted to Dr. M.S. Leung for teaching me the skill of dissection and for useful discussion, and Professor R. Linden for his advice during the writing of the manuscript.

I would also like to express my appreciation to Dr. S.B. Cheng-Chew and Miss Peggy Leung who have participated in the experiment on ultrastructural study and finally Miss P.Y. Lee for her encouragement during the study.

ABSTRACT

Functional characterization of slowly adapting type I (SAI) receptors in guinea pig hindlimb was carried out. All units were slowly adapting and possessed low force and displacement thresholds. They responded to mechanical stimulation in both the dynamic and static phases. The best fitting function of the force and displacement encoding ability was a linear and power function respectively. In response to a sustained stimulation the receptors usually showed 2 exponentially decaying phases of discharge. In its fully adapted state, the coefficient of variation approached 1 and the inter-spike-interval could be generally fitted by the Gamma-distribution. Transmission electron microscopy was used to verify the identity of the receptors studied, namely the Merkel cell-neurite complexes.

A possible role of phosphoinositide in the function of the Merkel cell was studied by neomycin and lithium administration. The effect of neomycin in guinea pig SAI receptors was studied by close arterial infusion of 9, 15 and 45 mg of neomycin into limb circulation. Neomycin produced a dose-dependent significant decrease in responsiveness of SAI receptors under mechanical stimulation (200ms ramp phase; 2s constant force of 20mN) applied every 30 seconds. Neomycin shifted the histogram

of ISI distribution towards longer intervals and cumulative percentage distribution showed significant differences in ISI samples obtained before and after neomycin infusion, under all three doses. Both the dynamic and static phases were affected.

The effect of Lithium on SAI receptors was studied in rats by chronic administration (intraperitoneal injection or by food intake). Afferent nerve fibres were sampled and single units recordings were made while SAI receptors were subjected to 40 consecutive mechanical stimuli (200ms ramp phase; 2s constant force of 15 or 20mN). Lithium produced a general reduction in responsiveness in Li-injected rats and the effect was most prominent under 15mN constant force. Both dynamic and static components were affected. Under a stronger stimuli of 20mN constant force, an increase in the first response to the trains of 40 stimuli was observed.

The findings support with the notion that phosphoinositide metabolism may play a role in mammalian SAI receptors.

INTRODUCTION

All mammals seem to possess Merkel cell-neurite complexes or functionally known as the slowly adapting type I (SAI) receptors. Although the functional characteristics and ultrastructure of the SAI receptors have been well known for some time, the mechanism of transduction and the role of the Merkel cell is still under controversy.

All environmental stimuli are encoded at the cell membrane into signals that elicit the appropriate physiological response. Recent evidence suggests that phosphoinositides which are located in the plasma membrane may constitute a fundamental transmembrane signaling system for a number of stimuli to cells such as neurotransmitters and hormones, and also sensory stimuli like sound and light.

The present experiments try to find out whether phosphoinositide metabolism may play a role in the functioning of SAI receptors by studying the effects of neomycin and lithium on the excitability of the receptors. Neomycin is ototoxic in nature and one of the possible action of its ototoxic effect is its specific interaction with the phosphoinositides. Since the guinea pig is a well studied animal model in ototoxicity, the species is used to test for the neomycin effect on SAI

receptors. The functional characteristics of the guinea pig SAI receptors have not been subjected to detailed study. Therefore part of the experiment involves the functional characterization of guinea pig SAI receptors.

Lithium has been used in a number of tissues as an enzyme inhibitor of the regeneration cycle of inositol. This feature of lithium is also employed in the present study to investigate the effect of its chronic administration on the responsiveness of SAI receptors in the rat.

SECTION 1. LITERATURE REVIEW

1.1 STRUCTURE AND FUNCTION OF SLOWLY ADAPTING TYPE I CUTANEOUS MECHANORECEPTORS IN MAMMALIAN SKIN

The mammalian skin has a rich innervation of afferent nerve fibres. According to their axonal diameter they are divided into large myelinated axons (Group II or Aβ), small myelinated axons (Group III or Aδ) and non-myelinated axons (Group IV or C). The group II axons innervate various types of mechanoreceptors. These include the Pacinian corpuscle, rapidly-adapting and slowly-adapting mechanoreceptors which have characteristic response behaviour to various patterns of mechanical stimulation (Figure 1). In mammalian skin, only two kinds of slowly adapting mechanoreceptors are known to be present. These are the slowly adapting type I (SAI) receptors and the slowly adapting type II (SAII) receptors which are correlated to the morphological structures of Merkel cell-neurite complexes and Ruffini endings respectively. They are described as slowly adapting because in addition to their response during the dynamic phase of mechanical stimulation, they also discharge during the static phase of sustained stimulation.

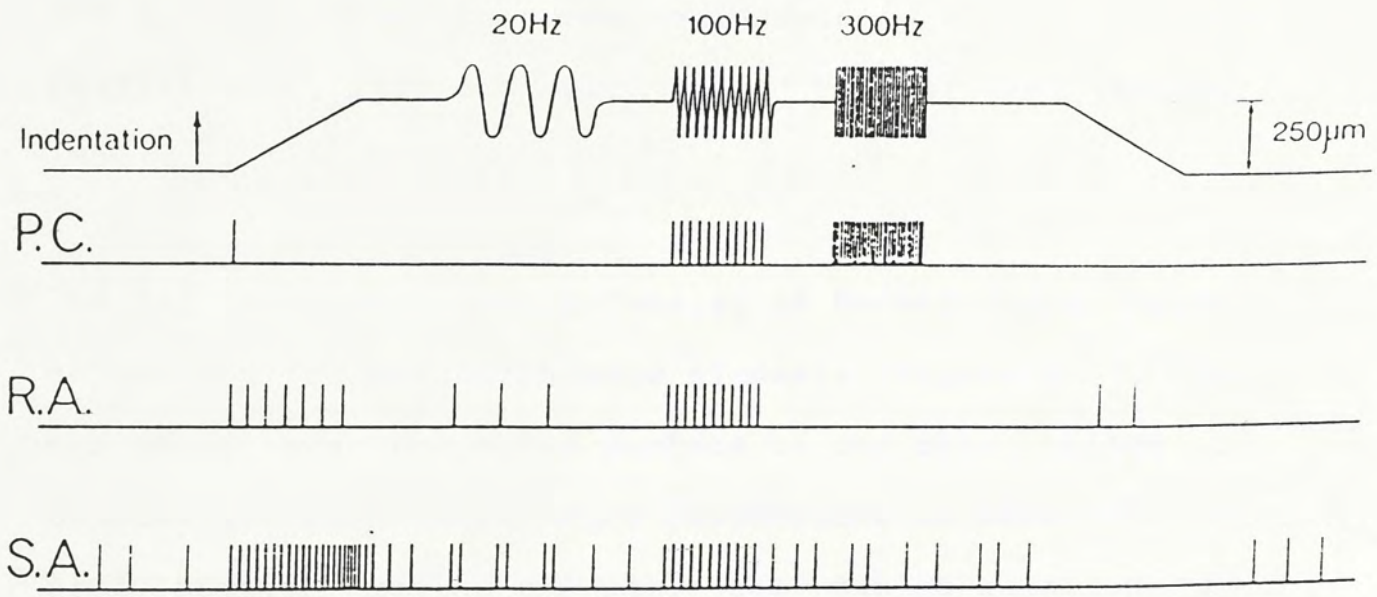


Figure 1. Diagrammatic illustration of the response of four afferent units to identical mechanical stimuli. PC,Pacinian Corpuscle; R.A., rapidly adapting receptor (Meissner, hair-follicle or Krause ending); S.A., slowly-adapting receptor (Merkel or Ruffini). (From Iggo, 1985)

1.1.1 Structure of SAI receptors

(a)Occurrence, Morphology and Morphogenesis

The SAI receptors or Merkel cell-neurite complexes can be found in both hairless (glabrous) and hairy skin. They are located either in normal epidermis or the sinus hair follicles (Iggo and Muir, 1969; Patrizi and Munger, 1966).

The SAI receptors, which consist of Merkel discs, Merkel cells and associated tissue elements (Figure 2), are scattered over the hairy surface of the skin, often in association with large hairs (tylotrichs of Straile). In some species such as the cat, they can be seen on the surface of the skin, where they appear as small dome-shaped spots. By using various microscopic techniques (Iggo and Muir, 1969), the morphology of the SAI receptors in the cats were studied. The epidermis of the dome is thicker than that covering the surrounding dermis, but the stratum corneum covering the elevation is thinner than of surrounding epidermis. On the crest of the dome, a single layer of Merkel cells lies on the epidermal side of the basement membrane. They have typical elongated nuclei oriented parallel to the surface. The afferent nerve branches as it approaches the epidermodermal junction, but the branches retain their myelin sheaths to within a short distance of the junction. Terminal branches then run towards the Merkel cells where an expanded nerve plate is associated with each Merkel cell.

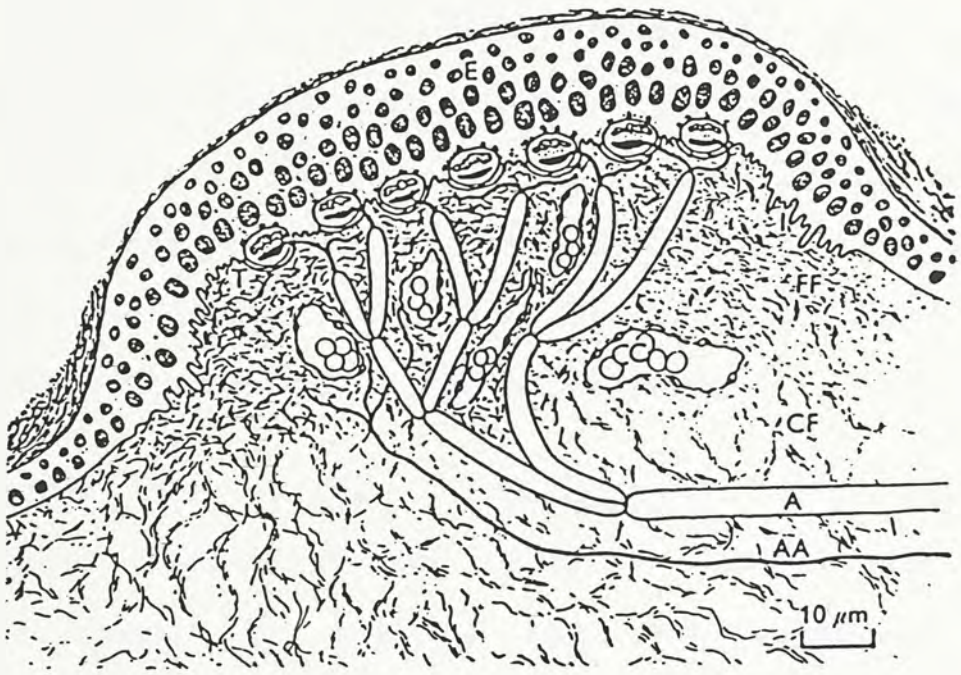


Figure 2. Diagrammatic representation of the structure of an SAI receptor (Merkel cell-neurite complex) as seen under light microscope. A, single myelinated axon; AA, nonmyelinated axons; E, thickened epidermis; FF and CF, fine and coarse bundles of collagen fibres; T, Merkel cell and its associated nerve plate; C, capillary (From Iggo & Muir, 1969)

The dermal core of the dome contains very fine collagen bundles woven into a three dimensional mesh and there is little extracellular fluid space. Arterioles and venules are not seen in the core of the touch corpuscles, but a convoluted plexus of capillaries permeates the dermis contained within the domes. No adipose tissue cells are present in the dermal core which has a normal complement of fibroblasts, mast cells and macrophages.

In glabrous skin the Merkel cells are at the base of intermediate ridges of the epidermis. Therefore the receptors cannot be seen at the skin surface. The receptors have typical Merkel cells and Merkel discs similar to those of hairy skins (Winkelmann and Breathnach, 1973; Munger and Pubols, 1972).

Sinus hair follicles are present in small numbers in the skin of many mammals. In a sinus hair, a uniform cylinder of Merkel cells surrounds the hair in the mid-region of the follicle below the sebaceous gland. The Merkel endings can number more than 600 (Halata, 1975) per hair.

There are currently two views concerning the origin of Merkel cells and both are supported by experimental evidence. One view is that Merkel cells are epithelial in origin. Evidence includes: 1)there exist cells in the epidermis of rats which are transitional in

appearance between Merkel cells and keratinocytes (English, 1977); 2)indirect immunofluorescence techniques revealed that Merkel cells contained keratin intermediate filaments and desmosomal protein (which demonstrated their epithelial nature) (Saurat, Didierjean, Skalli, Siegenthaler and Gabbiani, 1984; Ortonne and Darmon, 1985). Although this evidence supports the epithelial nature of Merkel cells, there are different opinions about the order of development. Lyne and Hollis (1971) found Merkel cells to be present within sheep epidermis during foetal development; but only in the oldest foetuses were these cells associated with neurites. Because the Merkel cells had desomosomal contact with adjacent epidermal cells, they concluded that they were modified epidermal cells. On the other hand, in newborn kittens, mechanically sensitive spots were innervated by type I afferent fibres even though very few Merkel cells were present (Kasprzak et al., 1970) This evidence suggested that Merkel cells differentiated from epithelial cells after the arrival of nerve fibres.

Another view concerning the origin of Merkel cells is that they are not epidermal in origin and that they may be derived from the neural crest. Relevant evidence includes: 1)apart from their typical location at the epidermo-dermal border Merkel cells have also been reported in the dermis of mammalian skin (Breathnach and

Robins, 1970; Breathnach, 1971a); 2)Breathnach (1971a) and Hashimoto (1972) both found Merkel cells present within the dermis and passing into the epidermis in tissue from human foetuses. 3)Winkelmann (1977) reported similarities between Merkel cells and amine precursor uptake decarboxylating cells (APUD) system and postulated that Merkel cells are migrants from the neural crest into the epithelium. Therefore at present the origin of Merkel cells is still uncertain.

(b)Ultrastructure

Figure 3 shows a diagrammatic structure of the Merkel cell-neurite complex under electron-microscopy. The Merkel cell-neurite complexes in hairy skin, glabrous skin or in sinus hair follicles possess similarities in their ultrastructure but minor differences with respect to orientation of the Merkel cell and surrounding tissue elements.

The Merkel cell and nerve terminal were first described electron-microscopically by Cauna (1962), and then by Iggo and Muir (1969). The terminal is an expanded and flattened disk (about 7 um in diameter and 1 um thick) and contains numerous mitochondria, which may occupy 50% of the terminal. Small, clear vesicles may also be present. The nerve plates may be borne on a single terminal branch, or they may be in tandem, i.e. the nerve

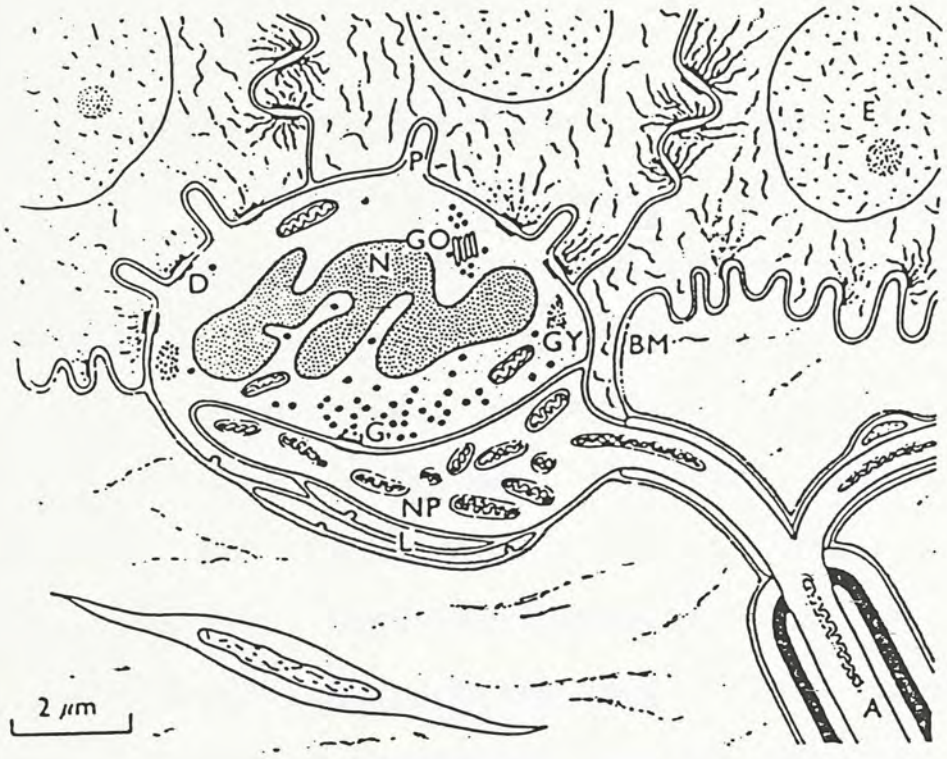


Figure 3. Detailed structure of a Merkel cell-neurite complex. A, myelinated axon; BM, basement membrane; D, desmosome; E, epithelial cell nucleus; G, dark-cored granules; NP, nerve plate; GO, Golgi apparatus; GY, glycogen; L, lamellae underlying nerve plate; P, cytoplasmic processes from Merkel cell (From Iggo & Muir, 1969).

forming an extension beyond one disc to form a further disc. When this happens the discs are close to each other (Iggo and Muir, 1969).

In adult mammals, the Merkel disc is always associated with a Merkel cell (Winkelmann and Breathnach, 1973).The characteristic features of Merkel cells are 1)a polylobulated nucleus which occupies a large part of the Merkel cell; 2)spherical, osmiophilic, membrane bound granules vesicles (84±13nm diameter) which are concentrated on the dermal side of the nucleus; 3)synapse-like junctions of the Merkel cell and nerve disc in which the cell membrane is flat and thickened. The nerve membrane is apposed to the cell membrane and a gap contains amorphous ground substances; 4)the Merkel cell contains a smooth endoplasmic reticulum in its superficial cytoplasm. The granular vesicles are probably formed in the region of the Golgi apparatus of the cell (Hashimoto, 1973) and stored on its dermal side; 5)the Merkel cell is attached to the adjacent keratinocytes by desmosomes, but the tonofibrils present are fewer in Merkel cytoplasm than in the keratinocytes. The attachments are absent from the typical finger-like projections of the Merkel cell which project into invaginations in the adjacent keratinocytes. These projections, 300nm in diameter and 2 to 3 um long (Iggo and Muir, 1969), are usually inserted into invaginations of the adjacent keratinocytes and rarely between them.

Other distinctive features are the absence of desmosomal attachments between finger-like projections and the enclosing keratinocytes, and the presence of longitudinally oriented filaments within them. However the nature of the filaments is still unknown at present.

1.1.2 Functional Characteristics

(a)General Properties

Detailed functional characterization of the SAI receptors in hairy skin has been performed in cat (Tapper, 1965; Iggo and Muir, 1969), rabbit (Brown and Iggo, 1967; Aitken and Lal, 1982) and rat (Leung, 1986). In general, the SAI receptor is characterized by a low mechanical threshold, absence of resting discharge and little or no response to stretching of the surrounding skin. These properties, in addition to the slowly adapting and irregularly discharging behaviour, are the criteria for the identification of the receptors (Horch et al., 1977).

1)Receptive Field Properties. The SAI receptors in the cat appear as small dome-shaped elevations of the epidermis and are therefore punctate in nature (Iggo and Muir, 1969). In the rabbit, careful exploration of the skin surface with a fine probe reveals that the receptive fields are not at all punctate: the threshold increases by 150% at a distance of 1mm from the centre of the dome;

at 2 mm it increases by 400% while at 3mm no response can be evoked.

2)Displacement and Force Threshold. The mechanical threshold of the SAI receptors in hairy skin is very low. For example, in the cat, the displacement threshold ranges from 1 to 5 um (Iggo and Muir, 1969). When measured by means of force, the threshold ranges from 0.02 to 0.1mN using von Frey hairs. In the rabbit, the displacement threshold, which was defined as an amplitude of stimulation at which 50% of trials evoked a response, ranged from 4um to 30um with a mean of 13um. The force threshold was not measured. In the rat, in which the mechanical threshold was determined in the gluteal region, the force threshold was reported to be 1.1 ± 0.1mN (Leung, 1986).

3)Resting Discharge. In the absence of external stimuli the SAI receptors are characteristically silent. The presence of a resting discharge is unusual and low in frequency. The receptors do not respond to stretching unless severe and prolonged (Chambers et al., 1972).

(b)Stimulus-Response Characteristics

In response to mechanical stimulation which consists of a dynamic component followed by a static component, the SAI receptors respond in several stages. There is a high frequency stage or dynamic response when the skin is

being indented. During the static response, the frequency initially declines rapidly and then more slowly. Similar stimulus response characteristic has been found in cat, rabbit and rat SAI receptors.

The frequency of the dynamic response is determined by both the velocity and the amplitude of displacement as shown in Figure 4. The amplitude is determined by the slope and the velocity is determined by the intercept. For constant final displacement, frequency or total count in dynamic phase relates to the velocity in a power function, in both cat (Iggo & Muir, 1969) and rabbit (Aitken & Lal., 1982).

The rate of adaptation is not a uniform phase and can be described by 2 time constants in the cat (Iggo and Muir, 1969) Under constant displacement, the units may eventually adapt to silence, but the total time during which a discharge is present varies from tens of seconds to minutes and also depends on the intensity of the applied stimulus.

(c)Analysis of Discharge Pattern

One of the diagnostic characteristics of SAI receptors is that the inter-spike-intervals (ISI) are quite variable. This is true even in early stages of adaptation, but particularly when the frequency has declined after

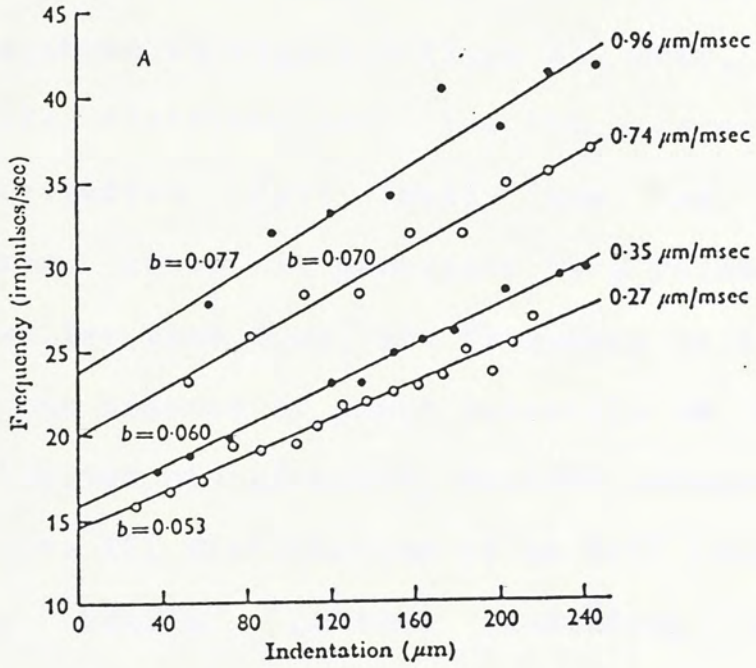


Figure 4. Dynamic response of a cat SAI unit during constant velocity indentation of the skin at the velocities indicated. Linear regression lines have been fitted to each response and the values of the regression coefficients (b) are shown on the graph. The responses depend on both the amplitude and velocity of the stimuli (From Iggo & Muir, 1969).

adaptation.

ISI histograms containing several hundred intervals such as the one shown in Figure 5 (Iggo and Muir, 1969) show a Poisson-like distribution. For ISI greater than 45ms, the distribution fits well into an exponential distribution, which is generated by a Poisson process. For ISI smaller than 45ms, the frequency is less and this reflects the absence of short intervals in addition to the contribution of refractory periods between spikes. In contrast, the ISI distribution of an SAII receptor shows a highly regular pattern resembling a Gaussian distribution or a noisy pacemaker . A useful parameter with which to measure the variability of ISI is the coefficient of variation (CV). In SAI receptors studied in cats the CV is greater than 0.5 whereas that of SAII is less than 0.3.

Studies from glabrous skin also show essentially similar patterns of ISI distribution. For example in the cat footpad, the irregular ISI produces a broad time interval histogram distribution with a high CV similar to that in hairy skin SAI receptors. A CV greater than 0.5 is common (Ferrington, 1985).

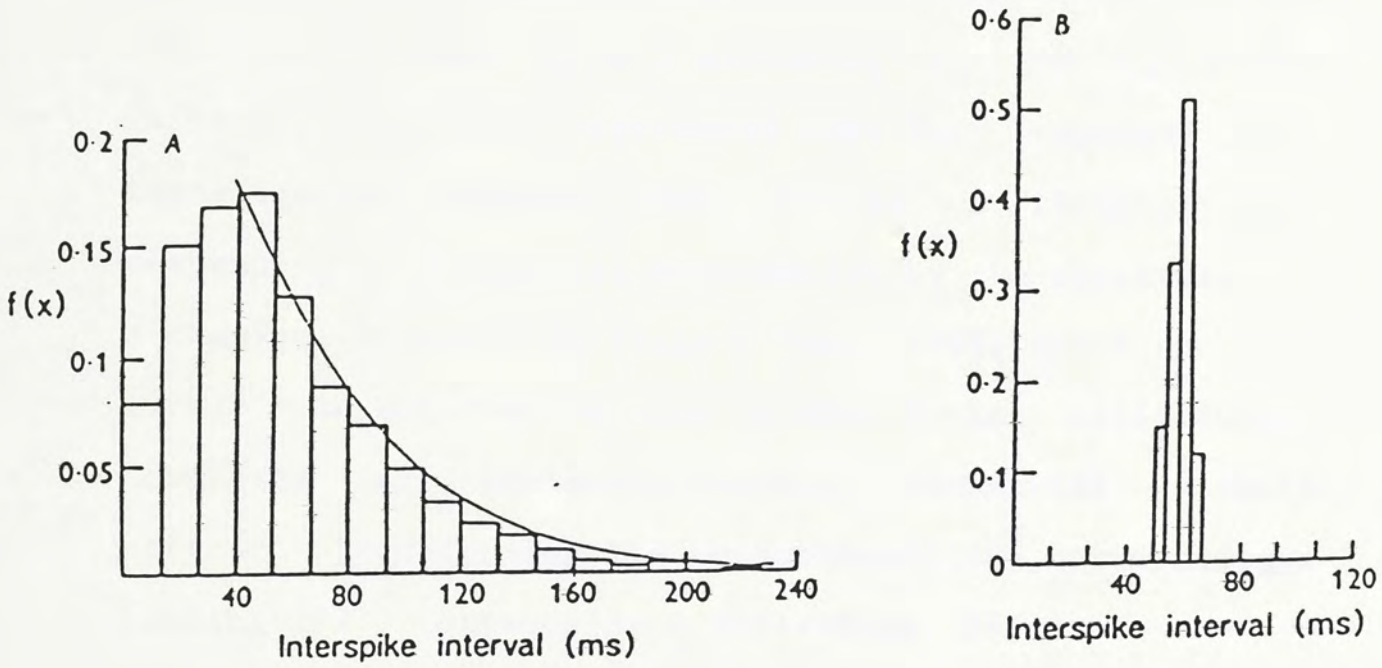


Figure 5. Typical frequency distribution of interspike intervals of cat SAI and SAII receptors. A. The adapted discharge for 1460 ISI. The theoretical density function $f(x)=pe^{-px}$ appears as a continuous curve, obtained after excluding the intervals shorter than 26ms, and where p=mean frequency. Coefficient of variation=0.63. B. Frequency distribution for the adapted discharge of an SAII receptor, with a mean frequency similar to the SAI receptor in A. Coefficient of variation=0.05 (From Iggo & Muir, 1969).

1.1.3 Transduction Mechanism

(a)Role of Merkel Cell

The functional role of the Merkel cell in the Merkel cell-neurite complex has been the focus of attention for some time. Based on both morphological and functional information, several hypotheses have been proposed. Some investigators suggest that it is a receptor cell responding to mechanical deformation by the secretion of a chemical transmitter (Iggo & Muir, 1969; Horch et al., 1974). An alternative view is that Merkel cell-neurite complexes are abutments making mechanical stimuli efficient in deforming the mechanosensitive nerve endings (Smith, 1977; Gottschaldt & Vahle-Hinz, 1981).

The evidence that favours a neurosecretory role of Merkel cells include: 1)Horch et al., (1974), from investigations to analyse the impulse discharge pattern, concluded that the most likely cause of the random discharging pattern was transmitter release from the Merkel cell; 2)ultrastructural studies suggest synaptic function; Chen et al., (1973) described in detail specialized junctions between Merkel cell and associated nerve terminals; 3)the presence of putative neurotransmitters such as vasoactive intestinal polypeptides (VIP)-like substances in Merkel cell (Hartschuh et al., 1983; 1984).

On the other hand, there is also evidence against a neuroceptive function of Merkel cells: 1)some authors found no evidence suggesting synaptic junction between the Merkel cell and the adjacent nerve terminal; 2)clear synaptic vesicles, which are a characteristic of synapses, are absent; 3)many drugs have been used to elucidate any neurotransmitter function of Merkel cells. Smith and Creech (1967) found that no drugs could elicit action potentials from the receptor. Many drugs which have an effect on known neurotransmitters such as acetylcholine and adrenaline had no effect on SAI receptors. For example, repeated reserpine administration had no effect on either the response or the ultrastructure of Merkel cell. Close arterial injection of histamine and acetylcholine, combination of histamine and acetylcholine and 5-hydroxytryptamine increased the response before depressing it (Fjallbrant and Iggo, 1961) but Chambers et al. (1972) later suggested that these responses were probably elicited from SAII receptors.

(b)Role of Dense-cored Granules and Calcium

In a different approach to determine the involvement of the dark-cored granules in the transduction process, Anand et al., (1979) found that hypoxia reversibly suppressed SAI responsiveness, which was accompanied by a

total loss of granules from the Merkel cell. The O_2-sensitivity can be explained by assuming there is a rapid uptake of neurotransmitter precursor, synthesis of the transmitter substance and its subsequent storage and release. Using the limb-perfusion techniques, Cooksey, Findlater and Iggo (1984) tested the importance of Ca2+ entry into cytosol to the response, and its relation to the lability of the granules. They found by introducing Ca2+-channel blockers, Co2+ and verapamil, that within a short time the SAI receptor failed to respond to mechanical stimulation. At the same time the afferent fibre still responded to electrical stimulation. Ultrastructural examination of the receptors which had been stimulated in the presence of the antagonists showed Merkel cells with a depleted number of granules.

(c)A Recent Model

By making an analogy between Merkel cell and hair cells of mammalian auditory system, Iggo and Findlater (1984) have proposed a model to account for the sequence of events during natural stimulation of an SAI receptor and this is summarized in Figure 6. The proposed sequences are: 1)mechanical distortion of filamentous rods of Merkel cells, 2)alteration of membrane permeability of epidermal surface of Merkel cell leading to 3)entry of Ca2+ into Merkel cell, 4)mobilization of osmiophilic granules, 5)release of granule contents at the synapse-

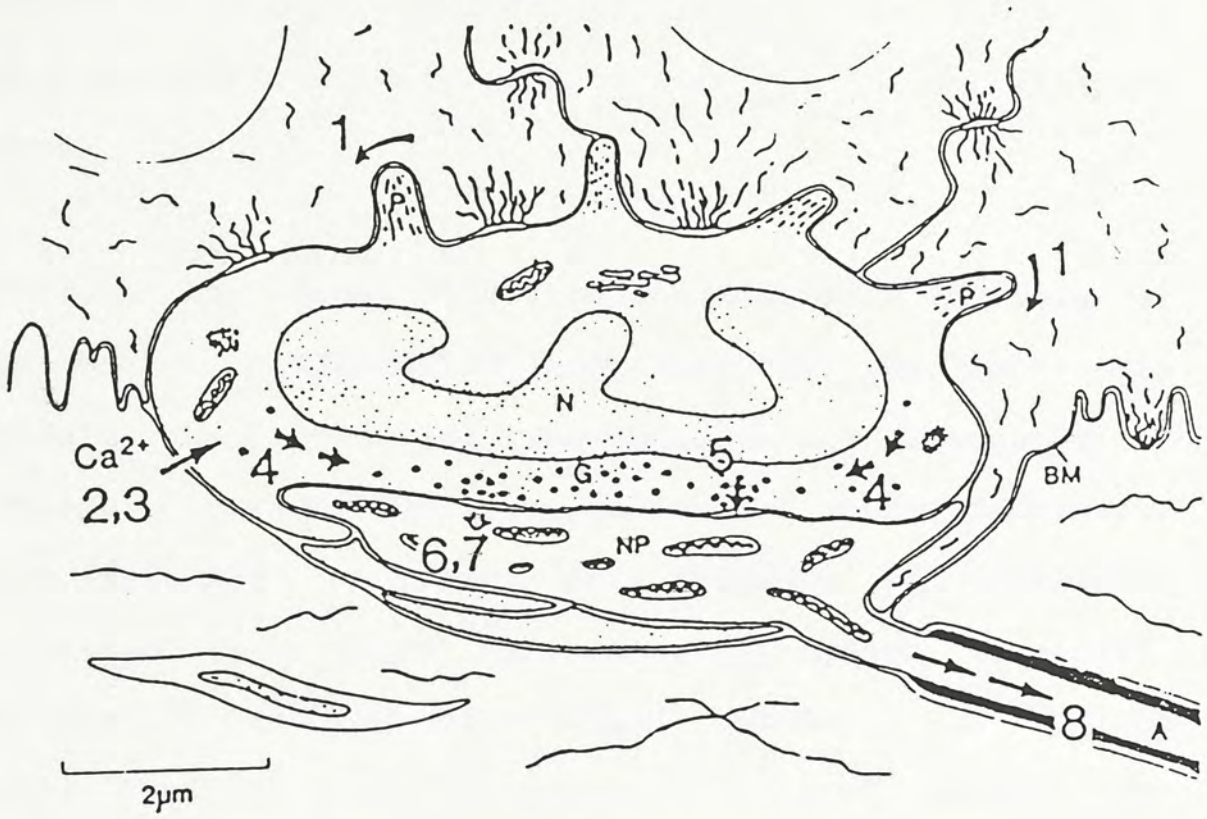


Figure 6. A Merkel cell-neurite complex showing sequences of events during transduction of SAI receptor, proposed by Iggo & Findlater (1984). The steps (1-8) are explained in the text.

like junction between the Merkel cell and the nerve plates, 6)alteration in permeability of nerve membrane leading to 7)the development of generator potential with the consequent, 8)initiation of impulses in the afferent axons.

Although the model discussed above defines the role of the Merkel cell and the importance of the dark-cored granules and Ca2+ during transduction, a knowledge of the process between the recognition of the mechanical stimulus and the entry (or release) of Ca2+ into cytoplasm is missing. Is a messenger system involved? At present the answer is still unknown. It is the purpose of the present experiments to test whether the phosphoinositides may play a role during this process.

1.2 ROLE OF PHOSPHOINOSITIDES IN SIGNAL TRANSDUCTION

1.2.1 Introduction

Studies on the transduction mechanisms of a number of hormones and neurotransmitters and even sensory stimuli like sound and light have led to the discovery that myo-inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DG) are important second messengers. These compounds are released by the hydrolysis of membrane phosphoinositides as a result of activation of the receptors by suitable stimuli.

The general structure of a phosphoinositol consists of 2 non-polar side chains of fatty acid and a polar head. They are acylated to the molecule glycerol. The polar head is the inositol and the fatty acid in the second position of the glycerol is usually arachidonic acid. Inositol is a sugar alcohol that can exist in several stereoisomeric forms. Myo-inositol is one which is naturally occurring. Phosphoinositides are a collective term to describe the three anionic phospholipids shown in Figure 7. The most abundant form is phosphatidylinositol (PI) that contains myo-inositol attached through the hydroxyl group in 1 position. The other two members are formed by sequential phosphorylation of the hydroxyl groups on the 4 and 5 positions of the inositol, which are called phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP2) respectively, and collectively called polyphosphoinositides (PPI).

These phospholipids are located in the plasma membrane of the cells and are thought to constitute agonist-sensitive pools in the cells.

1.2.2 Role in Pharmacological Receptors

In order for cells to respond to external signals such as hormones and neurotransmitters they must have mechanisms

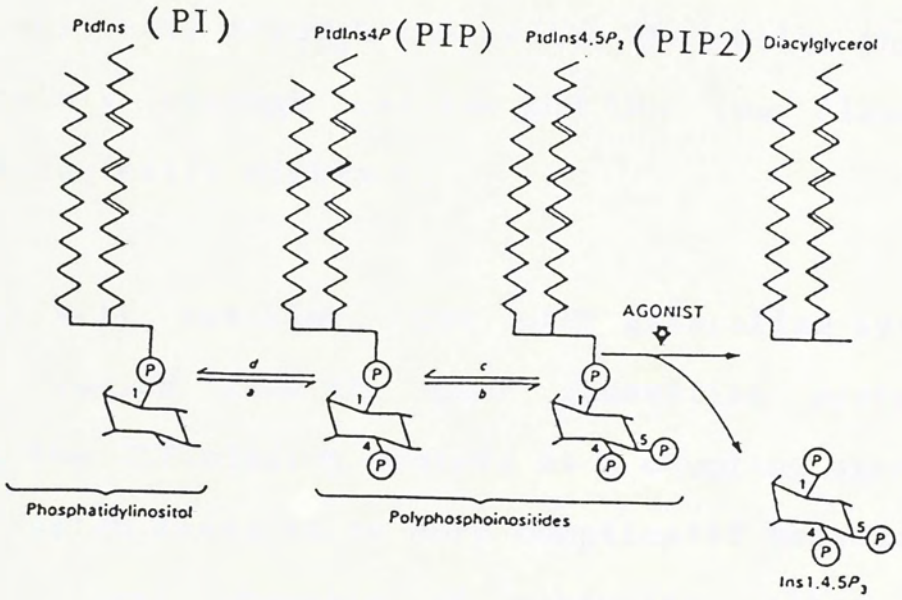


Figure 7. Structure of three phosphoinositides. PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate.

both for detecting and translating the incoming signals to the cell. Much of this translation occurs in the cell membrane by generating second messengers such as cyclic AMP (cAMP), cyclic GMP (cGMP) and calcium. There are two major classes of receptors for forming these messengers. One class acts through the cyclic AMP while the other acts mainly through calcium and the two classes are pharmacologically distinct.

Comparatively speaking, the cAMP generating system is better studied than the cGMP generating system. It employs the GTP-binding protein as a coupling agent. The other system seems to be more complicated because these receptors are capable of producing other second messengers in addition to calcium, such as cGMP and prostaglandins. Also, an early event associated with the activation of this group of receptors is the hydrolysis of the phosphoinositide PIP2.

(a)Properties of Receptors that Hydrolyse Phosphoinositides

A large number of different agonists can stimulate an increase in the metabolism of membrane phosphoinositides. These agonists include classical neurotransmitters such as acetylcholine (Weiss and Putney, 1981), noradrenaline (Uchida et al., 1982), histamine (Jones et al., 1979) and 5-hydroxytryptamine (Fain and Berridge 1979). In

addition, also included are more complex molecules such as vasopressin, (Rhodes et al., 1983), substance P (Weiss et al., 1982), angiotensin (Billah & Michell, 1979). The change in phosphoinositide metabolism is always specific for one particular class of receptors (Michell, 1975) such as muscarinic cholinergic receptor and the α1-adrenergic receptor. A common characteristic of the receptors is that they all are multifunctional in nature. That is, they have been implicated in the mobilization of calcium, for the activation of protein kinase C, for the release of arachidonic acid and the activation of guanylate cyclase (Berridge, 1981).

(b)Agonist-dependent Phosphoinositide Metabolism

Figure 8A shows the currently accepted scheme of the activation of an agonist-dependent phosphoinositide metabolism. The phosphoinositides are mainly confined in the inner leaflet of the plasma membrane with PI as the most abundant form. When the receptors are occupied, there is a rapid cleavage of PIP2 by a phospholipase C enzyme (phosphodiesterase enzyme) to give GP and IP3. The first indication that agonists hydrolyse PIP2 was obtained from studies on iris smooth muscle, where acetylcholine stimulated the breakdown of PIP2 (Abdel-Latif et al., 1977), leading to the accumulation of IP3. Such agonist-dependent hydrolysis of PIP2 has also been described in liver (Rhodes et al., 1983), parotid gland

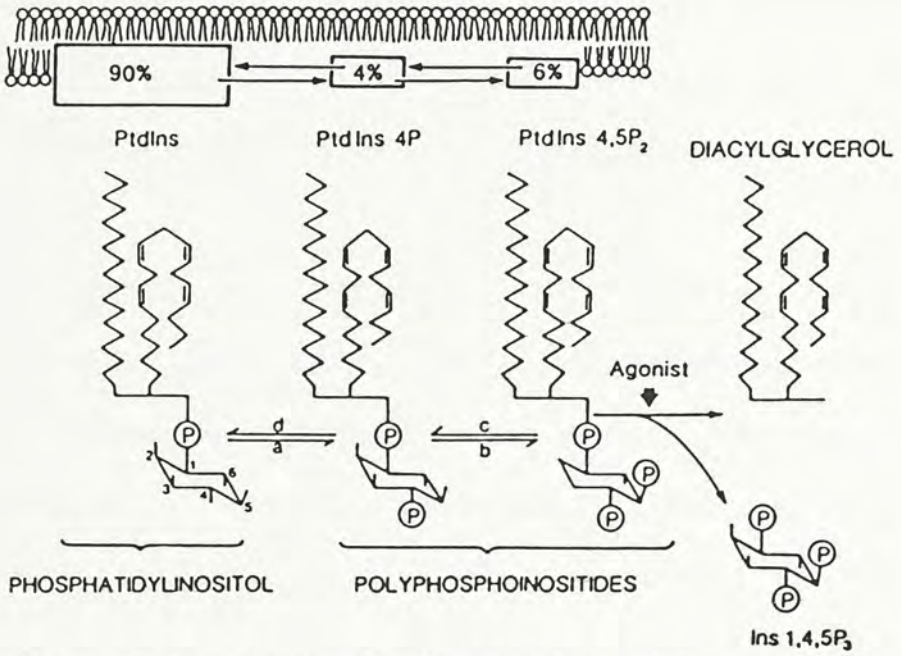


Figure 8A. A summary of the major biochemical pathway responsible for the metabolism of phosphoinositides. The boxes at the top represent the proportion of PI (PtdIns) and the two PPI (PtdIns 4P and PtdIns 4,5P2). Agonists act by stimulating the hydrolysis of PIP2 by a phosphodiesterase to give DG and IP3. a, PIkinase; b, PIPkinase; c,PIP2 phosphomonoesterase; d, PIP phosphomonoesterase.

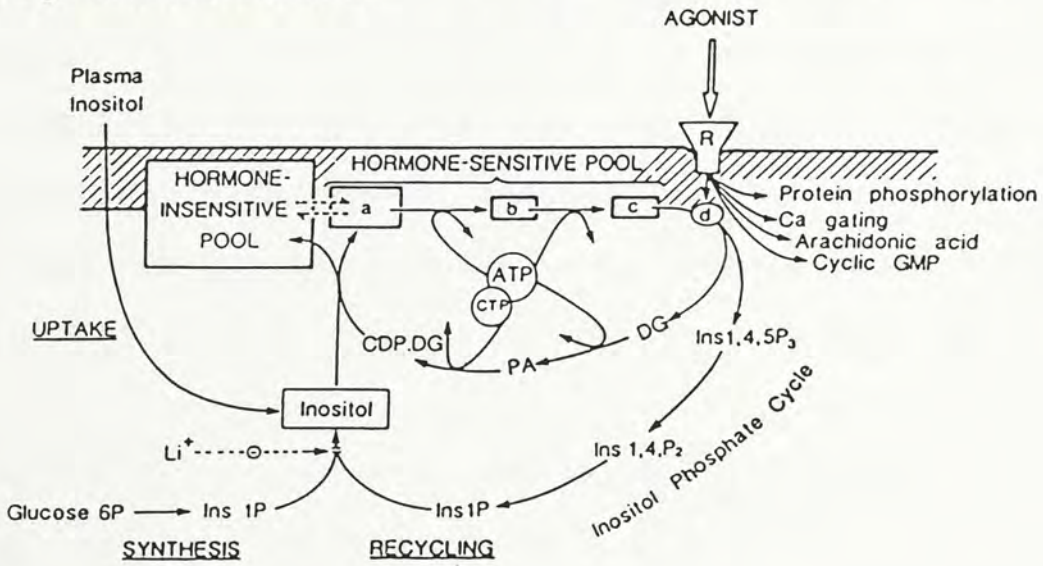


Figure 8B. Summary of the phosphoinositide metabolism and inositol phosphate cycle. (a)PI; (b)PIP; (c)PIP2; (d)phosphodiesterase; R,receptor. IP3 is dephosphorylated progressively to inositol when combines with CDP diacylglycerol (CDP.DG) to replenish the pool of PI. Inositol can also be taken up from plasma or it can be synthesized de novo from glucose-6-phosphate (From Berridge, 1985).

(Downes and Wusteman, 1983), blood platelets (Agranoff et al., 1983) and other tissues. The most convenient and sensitive method of monitoring this hydrolysis of PIP2 is to measure the formation of the two products.

The identification and measurement of inositol phosphates has provided an opportunity to study receptor events at very early time periods after the onset of stimulation (Berridge et al., 1985). When the salivary gland of the insect was stimulated with 5-HT, there was an immediate and rapid increase in the accumulation of IP3 and IP2. The increase in these two inositol phosphates was more than fast enough to account for the calcium-dependent physiological response in these glands (Berridge et al., 1983). Over these early time periods, there were no changes in the level of IP1 which is the expected product should PI be the substrate for the receptor mechanism. In keeping with rapid changes in the level of the inositol phosphates, there is an equally rapid increase in formation of DG (Martin, 1983;Thomas et al., 1983).

If DG and IP3 are to function as second messengers, it is essential that mechanisms exist within the cell rapidly to remove these internal signals once the external agonist is removed. Studies on the insect salivary gland have shown that IP3 level declines very rapidly towards its resting level when 5-HT is withdrawn (Berridge, 1983). An inositol trisphosphate phosphomonoesterase

that converts IP3 to IP2 has been found in human erythrocyte membrane (Downes et al., 1982) and insect salivary gland (Berridge et al., 1983). The latter also contains an inositol bisphosphate phosphomonoesterase that converts IP2 to IP. There thus seems to be an inositol cycle where the IP3 produced by the receptor mechanism is dephosphorylated in a stepwise manner to liberate the free inositol used to resynthesize PI. The final step in this cascade is the phosphorylation of IP to free inositol by the enzyme inositol 1-phosphatase (Figure 8B). There are two major ways for removing DG. It can either be converted to phosphatidic acid or it can be hydrolysed by a DG lipase to liberate arachidonic acid.

One interesting point about the agonist-dependent metabolism of phosphoinositides is that it is sensitive to changes in energy metabolism. The reason is that the recovery of PI from DG requires the expenditure of energy. ATP is used to make sure that 1)the signaling system not only responds to external stimuli rapidly but also 2)can maintain this responsiveness over an extended period of time.

(c)Inositol and Diacylglycerol as second messenger

The generation of the Ca2+ is the most important signal produced by these receptors. It was proposed that the

hydrolysis of phosphoinositides is responsible for the generation of Ca signal. The evidence suggesting such a relationship is as follows: 1)all receptors which hydrolyse phosphoinositides also appear to generate a Ca signal; so far there is no exception ;2)when the activity of PIP2-phosphodiesterase was studied using ionic conditions that matched the intracellular environment, there was little change in activity when Ca2+ was varied over its physiological range of 10-7 to 10-5M (Berridge, 1981); 3)similar observations in vivo where agonist-dependent breakdown of phosphoinositides occurred independently of changes in the cytosolic Ca^{2+}.

In order to obtain direct evidence for the idea that IP3 mobilizes intracellular calcium, various techniques have been used to make the plasma membrane permeable so that IP3 could gain access to the internal stores of calcium. For example, rat pancreatic acinar cells bathed in a culture medium responded to IP3 by a large release of Ca2+ (Streb et al., 1983). There was no release on addition of IP2 , IP or free inositol (Streb et al., 1983).

How can PIP2 breakdown regulate the Ca mobilization? There is no definite answer to this question. But there are a number of possible mechanisms that have been postulated. For example, it can act directly on internal

receptors to stimulate the release of Ca2+ or it can inhibit the mechanism responsible for the sequestering of cellular calcium (Berridge, 1981).

Diacylglycerol is responsible for the activation of the C-kinase which phosphorylates specific proteins in the cells and elicits other responses specific to the cells.

Recent studies suggest that a guanine nucleotide-dependent regulatory protein (G protein) couples PIP2-turnover dependent receptors to phospholipase C, a finding that presents a striking parallel with receptor regulation of adenylate cyclase. (Taylor & Merritt, 1986). As shown in Figure 9, when activated, the effector enzymes catalyse the formation of intracellular signal molecules, cAMP from adenylate cyclase, and IP3 and DG from phospholipase C. Cyclic AMP and DG activate their respective protein kinases, and IP3 mobilizes intracellular Ca2+. Together, the increased cytosolic Ca2+ concentration and phosphorylation of specific target proteins control the final cell response.

(d)Action of Lithium

The effect of Lithium ions on the metabolism of phosphoinositides was first noted when it was found that Li+ caused a reduction in the concentration of myo-inositol in the rat cerebral cortex (Allison & Stewart,

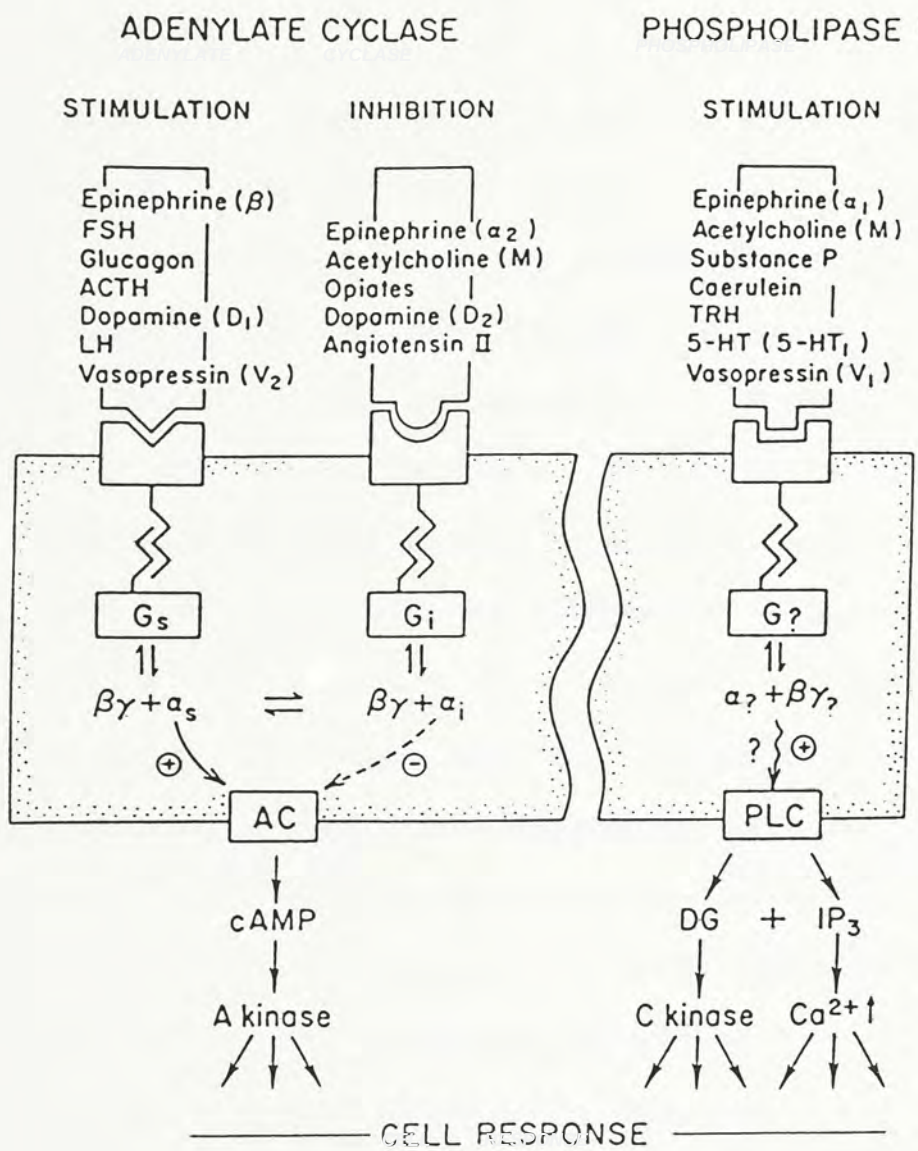


Figure 9. A parallel between receptor regulation of adenylate cyclase and phospholipase C activities. Interaction of an agonist with a specific cell surface receptor promotes dissociation of a specific G protein Gs for agonists that stimulate adenylate cyclase activity; Gi for agonist that inhibits adenylate cyclase; and G? for agonists that stimulate phospholipase C. Dissociated subunits of these G proteins then regulate intracellular effector enzymes; s stimulates adenylate cyclase and i may inhibit its activity; the means by which dissociated subunit of G? regulate phospholipase C are unknown (From Taylor & Merritt, 1986).

1971). Later it was found that accompanying this effect, Li+ also produced a marked elevation in the level of IP. (Allison et al., 1976).

In the mammalian tissues there are two known sources of IP: 1)the de novo synthetic pathway for myo-inositol formation, in which glucose is converted to IP; 2)from phosphoinositide metabolism via phospholipase activity, which produces D-myo-inositol. Phosphoinositide metabolism can also produce D-myo-inositol. The increased IP could thus be the result of an increase in phosphoinositide metabolism or synthesis or both.

To determine the source of the IP, it must be determined which enantiomer of inositol phosphates is affected, and the effect of Li+ on possible enzymes involved. It was not until 1981 that the problem of enantiomer was solved. By employing a gas-chromatographic system with an optically active liquid phase, the two enantiomers could be separated and it was found that chronic administration of Li+ elevates IP in which 90% belongs to the D-isomer. This suggests that the source of the myo-inositol comes from the breakdown of the phosphoinositides (Sherman et al., 1981).

In addition, a study on the IP phosphatase isolated from the mammary gland of rat showed that Li+ is highly inhibitory to the enzyme. However it was not known

whether this is also true for the nervous system. Later a detailed study on the enzymes isolated from bovine brain was carried out. Some important results concerning substrate specificity, effect of Mg^{2+} and effect of lithium have been studied (Hallcher & Sherman, 1980).

There are a number of substrates that can be hydrolysed by the enzymes. These include L-myo-inositol, (-)-inositol-3-P, D-myo-inositol and 2'AMP. The enzymes have a higher specificity for the L-isomer than the D-isomer. The enzymes also show an absolute requirement for the Mg^{2+} ion.

The effect of monovalent cations was also studied. Among the monovalent ions studied, Li^{+} ion is unique in that it is inhibitory to the enzymes while others are either slightly stimulatory or had no effect. Therefore the effect of Li^{+} is unique and is not a general effect of the group Ia elements. At about 0.8mM, 50% of the enzyme activities are inhibited. It was found that Li^{+} affected both the D- and L-isomers.

The discovery of the Lithium effect has some important implications for the study of the transduction mechanism. For example:

1) When free myo-inositol is decreased, the only other supply is plasma inositol. In the nervous system, in

both the CNS and PNS, it is more difficult to extract the myo-inositol. Therefore, these should be highly susceptible to the action of Li+.

2) This would explain the therapeutic action of Li+ on the manic-depressive illness because the pharmacological dose is of the same order of magnitude as that used in the in vitro studies.

3)The inhibitory effect of Li+ greatly amplifies the effect of the agonist. This enables us to study the response of brain cells to neurotransmitters. The data can supplement the studies by radioactive ligand binding.

1.2.3 Role in Sensory Receptors

In addition to the better established second messenger function of IP3 in cells responsive to hormones and neurotransmitters, there is evidence in recent years that IP3 also mediates the responses of the cells which respond to stimuli such as light and sound.

(a)Role in Photoreceptors

Studies from Limulus photoreceptors indicate that the cells contain the metabolic mechanisms necessary for the synthesis of the excitatory messenger, IP3. This is indicated by the findings that steady illumination

increased the incorporation of $^{32}PO4$ and 3H-inositol into phosphatidylinositol. Similar findings were found in acetylcholine stimulated $^{32}PO4$ incorporation into phosphatidylinositol in synaptosomes (Fisher & Amgranoff, 1981). In Octopus (Yoshioka et al., 1983), light caused a reduction of $^{32}PO4$ incorporation into polyphosphoinositides in a preparation of broken photoreceptor cells. Dark-adapted Limulus ventral eyes contained IP3 and the content of IP3 was increased by illumination.

Brief injection of IP3 solution elicited a depolarization that resembled the response of the cell to a flash of light (Brown et al., 1984). This caused a subsequent decrease in the sensitivity of the photoreceptor to light (Light, on the other hand, caused an apparent adaptation of the response elicited by IP3).

The effect of Ca2+ is similar to that of IP3. For example, Ca2+ injection desensitized the cells in a manner similar to that of light, whereas Ca2+ buffer EGTA tended to block desensitization of the cell (Fein, 1986). Illumination caused a rise in intracellular free Ca2+ concentration. These observations indicated that Ca2+ may also be an important component in the transduction mechanisms.

In Hemissuda photoreceptor, elevation of intracellular Ca2+ was shown to cause prolonged reduction of 2 voltage dependent Ca-currents across the cell membrane. Iontophoretic injection of IP3, but not IP, also caused prolonged reduction of the currents, which reflected its role in regulation of photoreceptor membrane currents (Sakakiba et al., 1986).

(b)Phosphoinositides in hair cell systems and the action of aminoglycosides.

Aminoglycosides are highly ototoxic. However, the mechanism of the ototoxic action is unknown. There are various possible explanations suggested. It has been suggested that aminoglycosides interfere with the active transport system essential for the maintenance of the ionic balance of the endolymph (Neu and Bendush, 1976); this leads to alteration in the normal concentration of ions in the labyrinthine fluids and leads to impairment of electrical activity and nerve conduction. On the other hand, aminoglycosides may have a direct plugging effect on the transduction channels in hair cells (Hudspeth, 1983; Ohmori, 1985) suppressing the transduction mechanisms. In addition to these observations, it has been found that the suppression effect on microphonic potential, or receptor current is partially restored by removal of the drug after a brief exposure (Brummet and Fox, 1982). This indicates that

the initial step of the ototoxic action may be on the surface membrane. Since aminoglycosides are strongly cationic compounds, they are expected to bind to negatively charged phospholipids.

Schacht (1974, 1976) found that neomycin affected the lipids of the cell membrane. He also demonstrated a high rate of polyphosphoinositide metabolism in the inner ear. Incorporation of labelled orthophosphate into the phosphoinositides of the organ of Corti was decreased by daily injection of neomycin parentally (Orsulakova et al., 1976). Yanagisawa et al., (1984) studied the effects of this aminoglycoside and calcium on the hair cell function and suggested that polyphosphoinositides (PIP and PIP2) may act as receptors for the aminoglycoside. Phosphoinositides and phosphatidic acid (PA) formation in the inner ear tissue of guinea-pig was tested in vitro. Sound stimulation enhanced their formation and neomycin suppressed it.

Kilian and Schacht (1981) reported similar findings in the auditory organ of the Noctuid moth in vivo. Stimulation with pulsed tones increased ^{32}P incorporation into PPI but not into ATP or other lipids (the effect was not seen when the stimulus was a continuous tone, leading to adaptation of the action potential). Interaction between aminoglycosides and PPI has also been measured directly by means of 31P nuclear magnetic

resonance. Calcium solution added to PIP2 solution moved the two monoester peaks while no change was observed in the diester group. Pretreatment with neomycin prevented Ca2+ from binding to any position (Sokabe et al., 1982).

In another study, monoclonal antibodies against PPI were raised in mice. When neomycin solution was applied to the antigen-antibody reaction medium, the reactivity between antibody and PIP2 or PA was reduced (Yanagisawa et al., 1984). All these data suggest that polyphosphoinositides are very important for sound reception and may have a function in the signal transduction system. However, up to present, there is no experiment on hair cells to study the effects of IP3 injections which may elicit responses similar to that found in photoreceptor cells.

The discussion so far has indicated that Li+ and aminoglycosides are two substances which have specific but different interactions on the metabolism of phosphoinositides metabolism in signal transduction; they have been used to study the signalling system in a number of tissues (Berridge et al., 1982; Carney et al., 1985; Cockcroft & Gomperts, 1985). These two tools have therefore been employed in the present study to investigate whether phosphoinositide metabolism is important in the transduction process of SAI receptors in the mammalian hairy skin. Section 1.3 gives a brief

review of the pharmacological properties of the two drugs.

1.3 PHARMACOLOGICAL PROPERTIES OF LITHIUM AND AMINOGLYCOSIDES

1.3.1 Lithium

Lithium is the lightest alkali metal (Group Ia). It is readily assayed in biological fluids by flame-photometric and atomic absorption spectrophotometric methods. Traces of Li+ occur in animal tissues but it has no known physiological role. It was introduced in late 1940 for the treatment of mania and is still an important drug in the treatment of this disorder.

Because of the importance of Li+ in clinical psychiatry, many of its pharmacological properties originate from human studies. The mechanism of action of Li+ as a mood-stabilizing agent remains unknown, although effects on biological membranes are suspected (Grafe et al., 1983). An important characteristic of the Li+ is that it has a relatively small distribution gradient across biological membranes, unlike sodium and potassium; it can replace sodium in supporting a single action potential in a nerve cell but it is not an adequate substrate for the sodium pump and therefore it cannot maintain membrane potentials.

Li+ is readily and almost completely absorbed from the gastrointestinal tract. In man, after an oral dose, complete absorption occurs in about 8 hours, with a peak concentration in plasma occurring 2 to 4 hours after administration.

Li+ is initially distributed in the extracellular fluid and then gradually accumulates in various tissues to different degrees. The final volume of distribution approaches that of the total body water (Baldessarini, 1985).

Passage through the blood-brain barrier is slow, but when a steady state is achieved the concentration of the Li+ in the cerebrospinal fluid is about 40% of the concentration in plasma (Baldessarini, 1985). There is no evidence of the ion binding to plasma proteins.

In man, the half-life of Li+ averages 20 to 24 hours; 80% of the filtered lithium is reabsorbed by the renal tubules. Na+-loading produces a small enhancement of Li+ excretion, but Na+-depletion promotes a clinically important degree of retention of Li+ that may lead to the development of toxic symptoms.

In man, because of the low margin of safety of the Li+ and because of its short half-life during the initial

distribution, divided daily doses are used in man, and even slow-release formulations are typically given twice daily. However, the necessity for such treatment instead of a daily single dose has been questioned (Plenge et al., 1981). In man parenteral administration is never employed.

Typically, transient mild polyuria appears early in treatment. Late developing polyuria is an indication to evaluate renal function. The mechanism of this effect may involve inhibition of the action of antidiuretic hormones (ADH) on renal adenylate cyclase, resulting in decreased ADH stimulation of renal reabsorption of water (Baldessarini, 1985).

The toxicity of Li+ is related to the plasma Li+ level and the rate of rise following administration. Acute intoxication is characterized by vomiting, profuse diarrhea, coarse tremor, ataxia, coma and convulsions. Symptoms of milder toxicity that most likely occur at the absorption peak of Li+ include nausea, vomiting, abdominal pain, diarrhea, sedation and fine tremor (Baldessarini, 1985). The more serious effects involve the nervous system and consist of mental confusion, hyperreflexia, gross tremor dysarthria, seizures and cranial-nerve and focal neurological signs, progressing to coma and death. (Saron and Gaind, 1973)

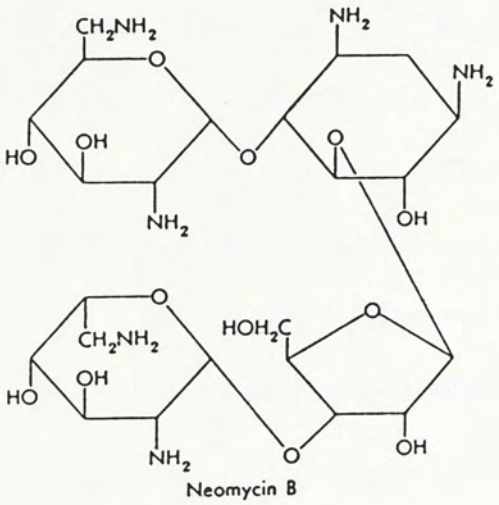
Treatment of Lithium intoxication by dialysis method indicates that when the concentration of Li+ in the plasma is lowered by dialysis or other means, recovery is still slow. This suggests that the intracellular concentration of Li+ may be the prime determinant of the appearance of clinical toxicity.

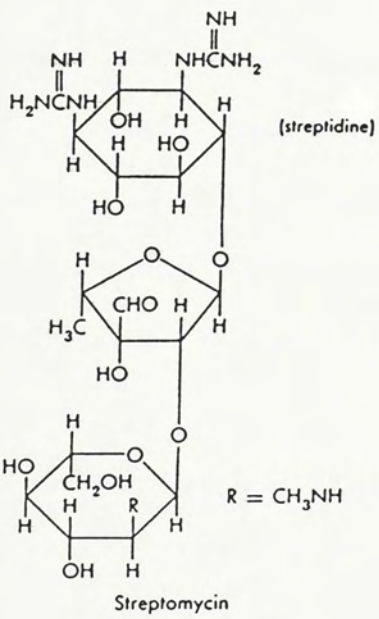
1.3.2 Aminoglycosides Antibiotics

All the aminoglycoside antibiotics, gentamicins, tobramycin, amikacin, netilimicin, kanamycin, streptomycin, and neomycin, contain amino sugars in glycosidic linkage and are polycations. The aminoglycosides are used primarily to treat infections caused by gram-negative bacteria; they act to interfere with protein synthesis in susceptible microorganisms (Sande and Mandell, 1985).

Serious toxicity is a major limitation to the usefulness of aminoglycosides and the same spectrum of toxicity is shared by all members of the group. Most notable are ototoxicity, which can involve both auditory and vestibular functions of the VIIIth cranial nerve, and nephrotoxicity.

Figure 10 shows the structure of 3 representative aminoglycosides of different structures: neomycin B,





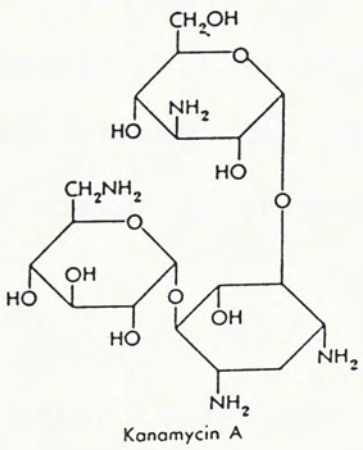


Figure 10. Structures of three representative aminoglycosides from different families. See text for details.

kanamycin A and streptomycin. Neomycin B and kanamycin are different from the streptomycin family in that their core sugars consist of 2-deoxystreptamine instead of streptidine. In neomycin B family, there are three amino sugars attached to the central 2-deoxystreptamine, which distinguishes them from the kanamycin and gentamicin families, which have only two such amino sugars.

The aminoglycoside antibiotics are rapidly bactericidal. While much is known about their mechanism of action at the ribosome, where they inhibit protein synthase and decrease the accuracy of mRNA translation, this does not explain their rapidly lethal effects. Currently it is suggested that progressive disruption of the cell envelope may explain the lethal action of aminoglycoside antibiotics (Bryan, 1984).

The aminoglycosides are highly polar cations; they are thus very poorly absorbed from the intestinal tract. Less than 1 % of a dose is absorbed following either oral or rectal administration. But all of these antibiotics are absorbed rapidly from intramuscular and subcutaneous sites of injection. Intramuscular injection results in peak concentrations in the plasma after 30 to 90 minutes (Sande and Mandell, 1985).

The aminoglycosides are largely excluded from most cells because of their polar nature. There is negligible binding

of aminoglycosides to plasma albumin. The volume of distribution approximates to the volume of extracellular fluid (Sande and Mandell, 1985).

Concentrations of aminoglycosides in secretions and tissues are low. High concentrations are found only in the renal cortex and in the endolymph and perilymph of the inner ear (Sande and Mandell, 1985); this presumably contributes to the nephrotoxicity and ototoxicity caused by these drugs. Some recent studies, however, demonstrated that an accumulation of gentamicin in the inner ear fluids over plasma levels does not occur (Huy et al., 1981) and Schacht (1986) argued that mechanism of ototoxicity should not base primarily on drug concentration reached in inner ear fluid.

The aminoglycosides are excreted almost entirely by glomerular filtration. A large fraction of a parentally administered dose is excreted unchanged during the first 24 hours, with most of this appearing in the first 12 hours. The half-lives of aminoglycosides in plasma are similar and vary between 2 and 3 hours (Barza and Scheife, 1977). The concentration associated with toxicity is not much greater than that required for treatment of many bacterial infections. The dosage is usually given in mg per kg body weight. Since aminoglycosides are minimally distributed in fatty tissue

the lean or expected body weight is always used.

All aminoglycosides including neomycin have the potential to produce reversible and irreversible vestibular and cochlear damage. Both vestibular and auditory dysfunction can follow the administration of any of the aminoglycosides. Studies of both animals and man have documented progressive accumulation of these drugs in the perilymph and endolymph of the inner ear (Huy et al., 1983). Ototoxicity is the result of progressive destruction of vestibular or cochlear sensory cells, which are highly sensitive to damage by aminoglycosides (Brummett and Fox, 1982). The biochemical mechanism for ototoxicity, however, is poorly understood. Early changes induced by aminoglycosides may be reversed by calcium.

The degree of permanent dysfunction correlates with the number of destroyed or altered sensory hair cells and is thought to be related to sustained exposure to the drug. Repeated courses of aminoglycosides, each resulting in loss of more cells, can lead to deafness. While streptomycin and gentamycin mainly produce vestibular effects, other aminoglycosides such as neomycin and kanamycin primarily affect auditory function (Sande & Mandell, 1985).

Unlike the effect on hair cells, the nephrotoxicity of

aminoglycosides is always reversible. Neomycin, which concentrates to the greatest degree, is highly nephrotoxic in man.

SECTION 2. METHODS

2.1 BASIC EXPERIMENTAL SETUP

The basic experimental setup consisted of three systems namely the mechanical stimulation system, the recording system and the data storage and processing system. Figure 11 is a block diagram representation of the whole system.

2.1.1 Mechanical stimulation

Mechanical stimulation was delivered to the skin using an electronic vibrator (Bruehl and Kjaer no. 4810). This, together with a stimulation probe attached to a force transducer (Statham UC2), served as an electro-mechanical transducer. The probe was made of nylon with a 1 mm diameter spherical tip. The force transducer monitored the force applied by the probe to the skin.

The displacement of the skin by the probe was monitored by an optical displacement transducer installed in the stimulator. Two modes of stimulation could be used: constant force and constant displacement. The control and measurement of the force and displacement were provided by an electronic feedback unit built in this university.

2.1.2 Recordings

Nervous activity was recorded using bipolar Ag-AgCl electrode hooks which were attached to a micromanipulator (Prior, England) to allow three dimensional fine adjustment of positioning. The action potentials generated by the receptor in response to mechanical stimuli were first amplified by a differential amplifier (Neurolog NL103), filtered (Neurolog NL125) and displayed on an oscilloscope with storage capacity (Tetronix 5103a). A spike processor (Digitimer D130) was used to give further amplification of the signals; it also allowed the setting of suitable discriminating levels observable on the oscilloscope. The force and displacement were also displayed on the oscilloscope. The Digitimer together with the function generator (Exact 336) was used to give different stimulation cycles of varying parameters such as cycle length, slope and duration of ramp phase etc. The Digitimer also sent trigger signals to different components.

Original recordings of nervous impulses from the differential amplifiers, and stimulation forces and displacements from the feedback unit were made with a 4-channel tape recorder (Hewlett Packard 3964A). Three FM channels were used to record force, displacement and nervous activity and a direct channel was used to record the human voice with remarks on the experimental

procedure and trigger pulses from the Digitimer.

The chart recorder (Graphtec WR3101) was used to record cumulative spike counts (from Digitimer spike processor) during individual stimuli, displacements, forces and arterial blood pressure. The blood pressure was amplified through a recorder amplifier (Neurolog NL107). Another oscilloscope (Philips PM3230) was used to monitor the signals from the tape output.

2.1.3 Data Processing

A 6502-based microcomputer was used for data processing. Analog values representing force, and displacement (from feedback unit), and cumulative counts (from Spike Processor) were digitized by an Issac Analog-to-Digital (A/D) converter. The data were processed by computer programs written in Applesoft Basic and the values of force, displacement and nervous response were displayed on-line. The raw data were stored on floppy disks which allowed statistical analysis to be carried out later. The magnetic tape could also be replayed so that further analysis of the data such as ISI distribution was always possible.

2.2 FUNCTIONAL CHARACTERIZATION OF THE SAI RECEPTORS IN GUINEA PIG

2.1.1 Surgical Preparation

Experiments were performed on eleven guinea pigs of both sexes weighing 650g to 900 g. Each was anaesthetized initially with urethane (30% w/v, 3 ml/kg, i.p.). Supplementary doses were given intravenously as required. One carotid artery and one jugular vein were cannulated allowing the recording of arterial blood pressure and venous access respectively. The minimum mean blood pressure allowed was 50 mmHg as the normal blood pressure of guinea pig is characteristically low (Sisk, 1975). The body temperature was kept at 37 ^{0} C by a thermostatically controlled electric blanket. The hair on the right hindlimb was shaved closely with a clipper without using depilating agent. The right sapheneous nerve was exposed and the skin on that area was sewn to a lead ring, and the nerve was covered by a pool of warm paraffin oil. Under a dissecting microscope (Zeiss OPM1-1F) the nerve fascicles were desheathed and small strands of nerve dissected, placed on the electrodes, and tested for slowly adapting afferent units.

Since the characteristic elevated domes that can easily be seen in other species such as cats and rats are not so distinct in the hairy skin of guinea pig, this criterion,

which is regarded as a reliable one, cannot be employed in the present study. Receptors were initially identified by their slowly adapting behaviour during sustained stimuli, little or no response to mild stretching and by their responsiveness to stimulation of only small spots of skin (Hamann & Lee, 1982).

2.2.2 General Properties

After a receptor has been identified, the following parameters were determined for each unit: (1)Displacement threshold: by applying a standard slope of indentation of 0.01mm/ms, the displacement at which the first impulse appeared was taken as the displacement threshold; (2)Force threshold: by applying a standard slope of increasing force of 0.1 mN/ms, the force level at which the first impulse appeared was taken as the force threshold; (3)Surface morphology: under the dissecting microscope it was observed whether a clearly visible dome can be seen; (4)On-going activity: if present, the resting discharge of each receptor was noted; (5)Response to stretch, if any, was tested by stretching of the skin across the receptors. Mild to vigorous stretching was applied and directional sensitivity was determined.

2.2.3 Stimulus Parameters and Statistical Analysis

The ability of the receptors to encode for different magnitudes of dynamic and static stimuli were studied by standard stimuli of varying plateau force levels. Each individual stimulus rose over 200 ms to a force of 5, 10, 15 or 20 mN and the force was maintained for 2s, the interstimulus time being 30 s. Several stimuli (usually 5 identical ones) were taken for each pattern of stimulation. The receptors were allowed 5 minutes between each run. The amplitudes required to maintain the force levels were also noted.

In order to study the adaptation characteristics of the receptors, including the analysis of ISI distribution in the fully adapted state, 12 units were given their maximal constant displacement (1.5 or 2mm) stimulation for 30s, maintaining the standard dynamic 200 ms duration at the beginning of the stimulation. The rates of adaptation were characterized by estimating the time constants of the exponential decrease of discharge frequency with time.

Analysis of the variability of the generation of action potentials or the ISI distribution was carried out. The post-stimulus time (PST) histogram data was obtained by replaying the magnetic tapes. The information of ISI distribution was then extracted from the PST histograms

data using another program. The period used for the analysis of ISI distribution was the fully adapted state, usually obtained between 10s and 30s after the onset of stimulation. ISI histograms for several identical stimuli to the same unit were obtained for statistical analysis. The total number of intervals ranged from 266 to 1245.

2.2.4 Histological Studies

After each experiment skin samples containing putative SAI receptors were taken from the hindlimbs and were fixed immediately in a 4% gluteraldehyde solution which was buffered with Millonig buffer at pH 7.3. The specimens were then trimmed into smaller pieces to about 1-2mm in length. After fixation with 2% osmic acid the preparation was placed in Millonig buffer for 2 hours during which several changes of buffer were made. Dehydration was then carried out in a series of up-graded alcohols. The specimens were finally embedded in Spurr medium. Polymerization was carried out overnight in an oven at 60°C.

Thick sections of 1-2um were obtained by cutting the blocks with a Reichert ultramicrotome. They were stained with filtered Toluidine Blue solution for identification and localization of Merkel cells. Some specimens were then further trimmed down for electron microscopic study. Gold and Silver thin sections were cut with a diamond

knife (Diatome) and mounted on clean copper grids. Double staining with uranyl acetate and lead citrate was carried out each for 30 minutes. Sections were examined with a Joel 100CX2 electron microscope.

2.3 EFFECTS OF CLOSE ARTERIAL ADMINISTRATION OF NEOMYCIN IN GUINEA PIG

2.3.1 Surgical Preparations

Acute experiments were performed on guinea pigs of either sex (690g - 900g). The surgical procedure was the same as in 2.1.1. but in addition, a branch of the femoral artery serving areas other than the limbs was cannulated in a retrograde fashion to allow slow infusion of fluid into femoral artery without substantial change in femoral blood flow. The latency of the system was 8 minutes.

From bundles of desheathed saphenous nerve, small nerve strands containing single afferent units from SAI receptors in the skin of the antero-lateral aspect of the lower leg were dissected. Receptors were identified by the following criteria: 1)slowly adapting response to a sustained stimulus; 2)no response to mild stretching; 3)localized receptive field and 4)irregular discharge to sustained stimuli.

2.3.2 Stimulus Parameters

Standard mechanical stimuli comprising a 200 ms ramp and 2s plateau phase of 20 mN constant force were applied every 30 s. Between stimuli a contact force of 0.2 to 0.5mN was maintained, which was below threshold for a static response for most SAI receptors. During control periods normal saline was infused into the artery via an infusion pump (Harvard) at a rate of 0.026ml/min. During test periods the same volume was infused delivering a total of 15 mg of neomycin as sulphate (Myciradin Upjohn) in 5 minutes. For 2 guinea pigs a lower dose of 9mg was given and for another 2 a higher dose of 45mg was given to test the effect of different doses of neomycin on the receptors. Only one receptor was investigated in each animal.

2.3.3 Statistical Analysis

Comparisons were made between the following, before and after infusion of neomycin:

1)Nervous response: the total counts, nervous response in the dynamic phase and plateau phase.

2)Compliance of the skin: the residual indentation, maximal indentation and stroke amplitude required to maintain the same constant force.

3)ISI distribution during 2 phases of stimulation period: dynamic phase (0-250ms) and static phase (1200-2200ms).

All results are expressed as mean±S.E.M. and comparisons were made with Student's t test using a significance level of 0.05.

2.4 EFFECT OF CHRONIC ADMINISTRATION OF LITHIUM IN RAT

2.4.1 Chronic administration and monitoring of Li+-level

Two modes of lithium administration were used in order to compare the effects of different means of administration of lithium: 1)By food and 2)By i.p. injection.

1)By food

Rat food chow with an average content of 60mmoles/kg Li+ was prepared by first dissolving LiCl salt in water. The chow was then mixed with this solution and then dried in an oven. Control rats (N=5) received food chow prepared in the same way except that the same volume of distilled water was used instead of LiCl solution.

Such a treatment leads to a rather constant Li+ serum level during the second week in the rats averaging 0.9±0.2 mM measured by flame photometer (Corning 435). The blood samples were obtained from the orbital sinus. Rats (N=5) treated for 2 weeks were subjected to acute

experiments.

2)By I.P. Injection

Animals (N=5) were given LiCl of 5 mmole/kg body weight, in 1 ml of distilled water, i.p. for 2 weeks every morning. This resulted in mean plasma Li+ values of 1.9±0.1mM at the end of experiments (an average of 8 hours after the last injection). Control animals (N=6) were treated in a similar way except that LiCl was replaced by NaCl similar to that in control rats.

Care was taken to avoid the development of toxicity which was characterized by a distinct decrease in body weight accompanied by polyuria and polydipsia. Therefore, the body weight, water and food consumption were recorded every day for all the rats under investigation.

2.4.2 Mechanical Stimulation and Recordings

Identified receptors were subjected to trains of 40 mechanical stimuli, each comprising a 200ms ramp rising phase and 2s plateau phase. The plateau force was maintained at 15mN or 20mN. The interstimulus interval was 250ms during which 0.5 mN force was applied. This pattern of stimuli was adopted to produce a rather exhausting stimulation to the receptor in order to study the adaptation behaviour of receptors. Sufficient time

(12 minutes) was allowed for the receptor to recover before another train of stimuli was given. An average of 2 to 3 trains were given to each receptor.

Nervous discharge to stimulation, force of stimulation and resulting displacement were recorded.

2.4.3 Statistical Analysis

Comparisons between control rats and treated rats were made of the following:

1)Nervous response: the total counts and the responses during the dynamic phase and the static phase.

2)Compliance of the skin: the residual indentation, maximal indentation and the stroke amplitudes required to maintain the same constant force.

All results are expressed as mean±SEM and comparisons were made with Student's t test using a significance level of 0.05.

SECTION 3. RESULTS

3.1 FUNCTIONAL CHARACTERIZATION AND ULTRASTRUCTURE OF GUINEA PIG SAI RECEPTORS

A total of 20 units on the lateral aspect of the lower leg were studied. They all exhibited slowly adapting behaviour and had irregular discharge. All of them were subjected to the general tests and 12 of them allowed more detailed analysis on stimulus-response characteristics.

3.1.1 Functional Characteristics

(a)General Properties

The units possessed very low dynamic mechanical force and displacement thresholds. For the 20 units examined the force threshold (under the given standard force slope of 0.01mN/ms) was less than 1 mN. The displacement was less than 25um.

Only 4 out of 20 showed the appearance of clear elevated domes. This is in contrast to the observations on cat, rabbit or rat. The difficulty in the observation of distinct domes may be due to the undulating nature of the skin surface observed after removal of the hairs without depilation.

Most of the units did not exhibit a resting or on-going discharge in the absence of applied stimuli. Only 4 units showed a low frequency (<0.25Hz) irregular discharge. In response to mild stretching, most of the units did not respond. However, under vigorous stretching more than half of the units responded and some had directional preferences.

(b)Stimulus-response Characteristics

In response to standard stimuli comprising a dynamic phase of 200ms and a plateau phase of 2s, the units responded with a high frequency discharge in the dynamic phase and the early part of the plateau phase Figure 12 shows a typical recording of the nervous activity, resultant force on the skin and displacement of the probe stimulating the receptor. The pattern is similar to that obtained from SAI units in hairy skin of other mammals. Analysis of the ISI distribution during the dynamic phase indicated that the discharge frequency can be up to 1 kHz (minimum ISI of 1 ms).

Six units were tested with respect to their force response characteristics in the range of 5 to 20 mN. Figure 13 shows the average result of the units investigated. The nervous activities during the dynamic phase and static phase are linearly related to the final

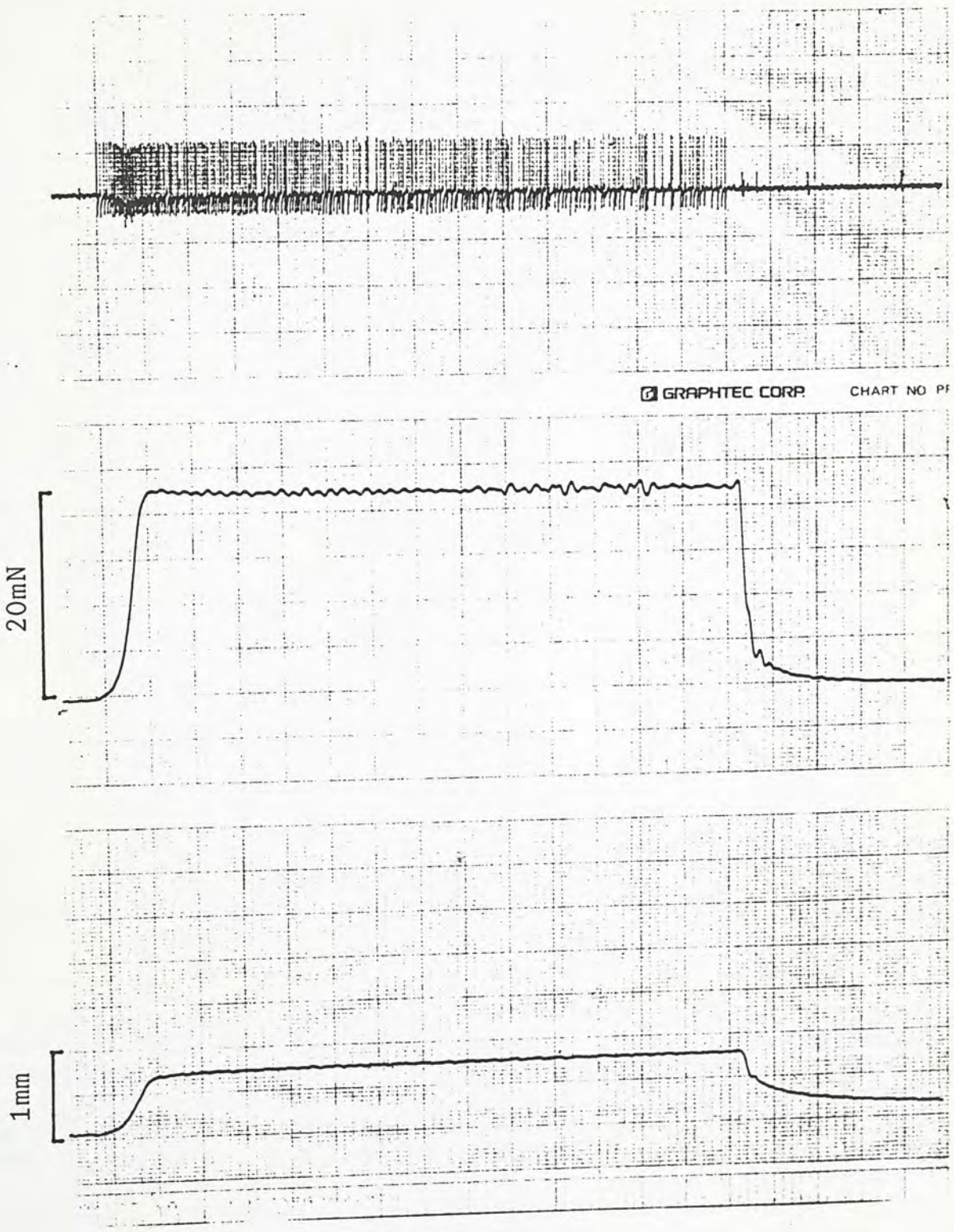


Figure 12. Original recording of the nervous activity (upper trace), applied force in mN (middle trace) and the displacement of the probe (lower trace) of a typical mechanical stimulus consisting of 200ms ramp phase and 2s constant force (20 mN) static phase applied to an SAI receptor in the hairy skin of guinea pig.

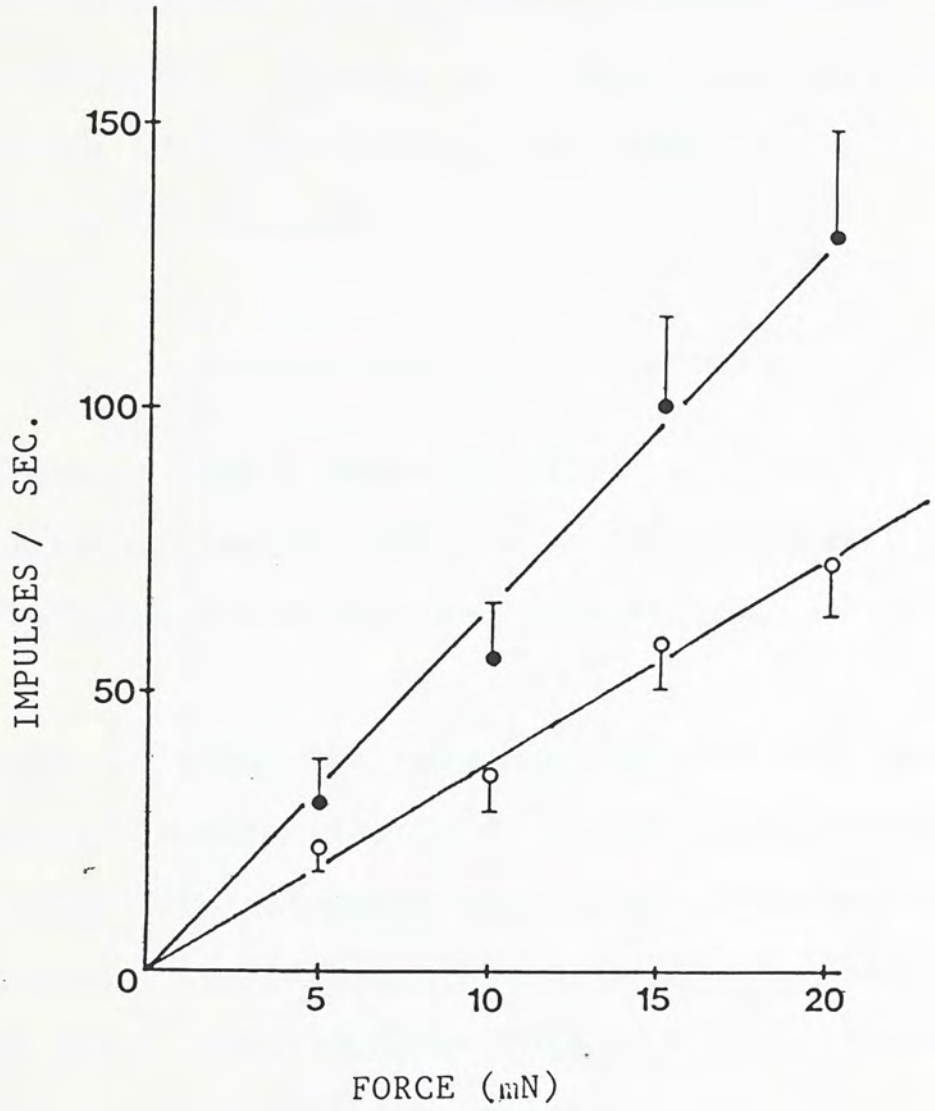


Figure 13. Relationship of frequency of impulses generated in the dynamic and plateau phase with the force applied to SAI receptors of guinea pig. Each stimulus consists of 200 ramp phase and 2 s constant force (5, 10, 15 or 20mN). The dynamic response (●) and the static response (○) are linearly related to the plateau force values. Each point represents the mean response of 6 units tested and vertical bars represent SEM of the means.

plateau force amplitude (r=0.996 and 0.995 respectively).

The relationship of the force and displacement was also investigated. Figure 14 shows that displacement was related to force (during 5 to 20mN) by a power function given by the formula

$$\text{Displacement} = A \times \text{Force}^{N}$$

whereas A and N were constants with N<1. Regression analysis showed that this relationship gave best correlation (r= 0.999) and that N=0.53.

Figure 15 plots the responses against the displacements required to maintain the different levels of forces; the resultant relationship was a power function in which the exponent N was greater than 1. Regression analysis showed that N=1.6 with r=0.986. This is a logical result of the first two relationships explored.

The adaptation behaviour of the SAI receptors was analysed for the first 25 s of maintained constant displacement stimuli using a binwidth of 200ms. Figure 16(a) shows a typical response of an SAI unit during the 25s stimulus period. The results were plotted to a semi-log graph in Figure 16(b) in which two negative exponential phases were apparent and which could be characterized by 2 time constants, 1.9s and 134.5s

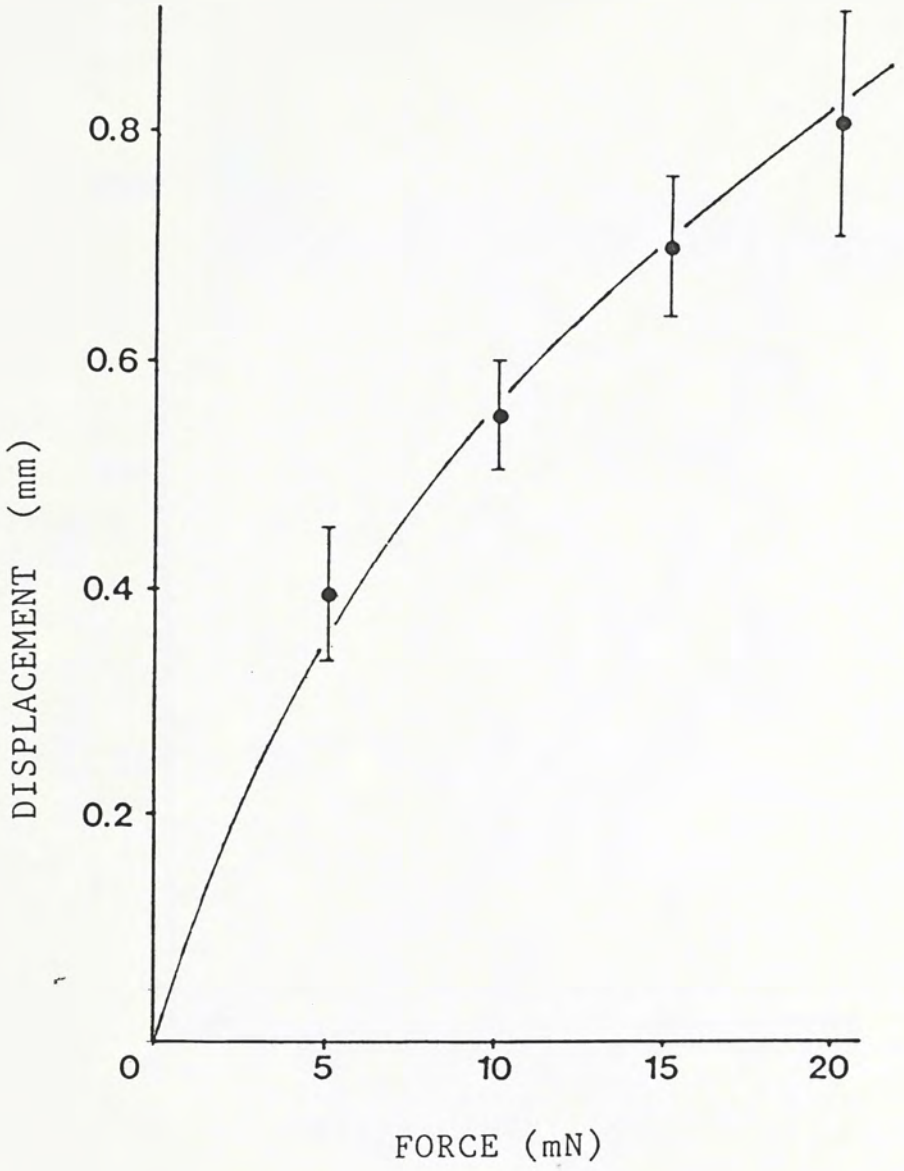


Figure 14. Displacement-force relationship of the skin for various force amplitude applied to SAI receptors of guinea pig. Each stimulus consists of 200 ramp phase and 2s constant force (5, 10, 15 and 20 mN). The displacements are values required to maintain the constant forces. Each point represents the mean response of 6 units and vertical bars represent SEM of the means.

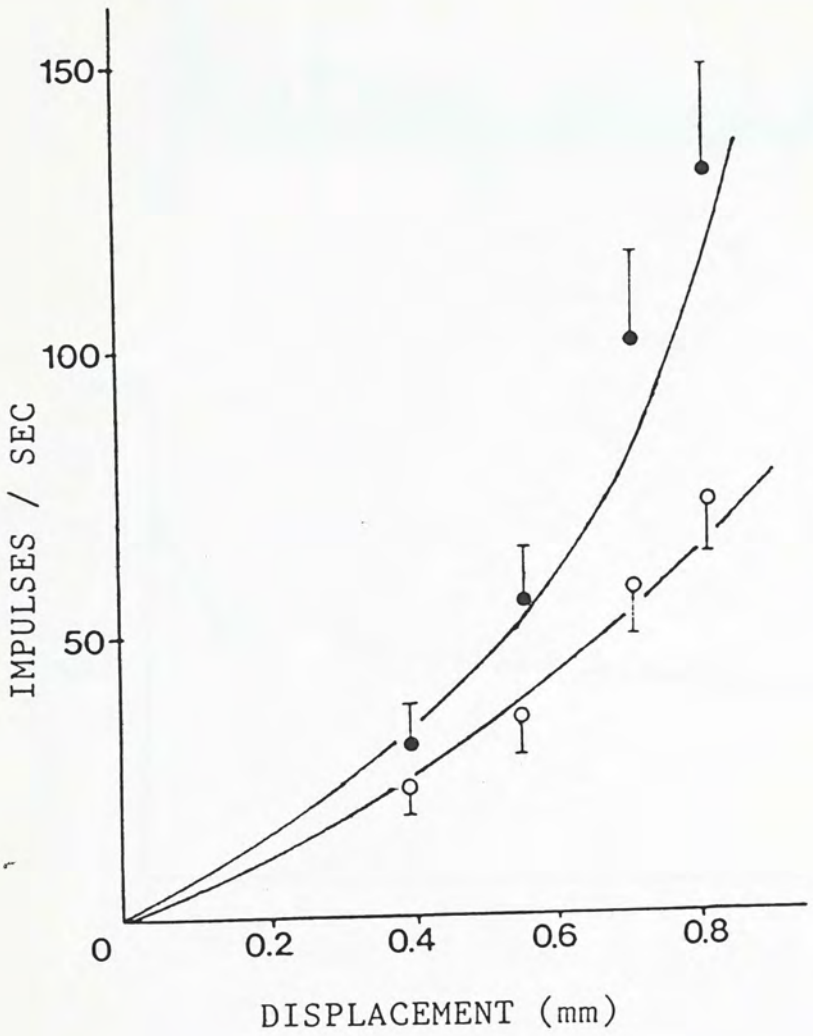
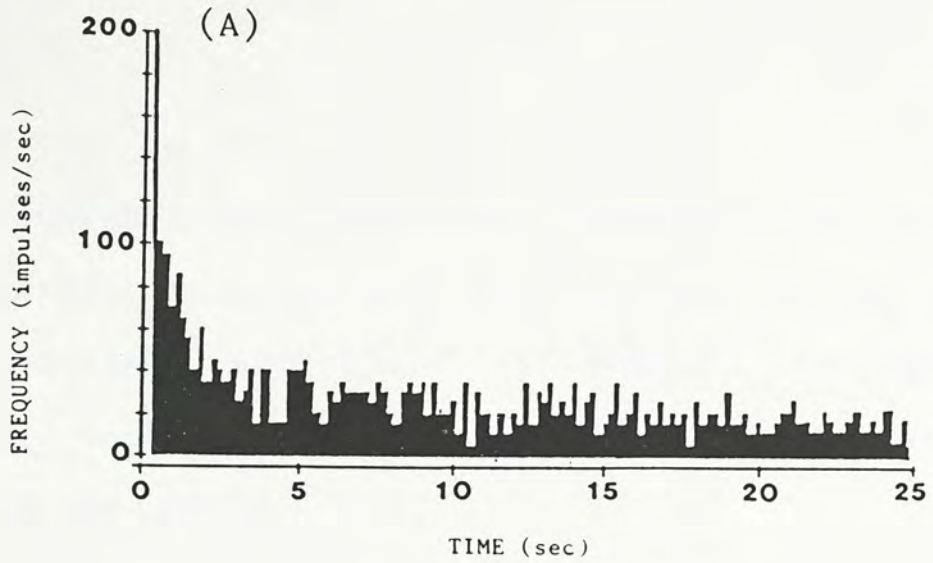


Figure 15. Relationship between the frequency of impulses generated with the final displacement values required to maintain various constant force levels. Each stimulus consists of 200ms ramp phases and 2s constant force (5, 10 15 and 20mN). The dynamic (●) and static (○) response are related to the displacement with a power function where exponents = 2.2 and 1.7. Each point represents the mean response of 6 units and vertical bars represent SEM of the means.



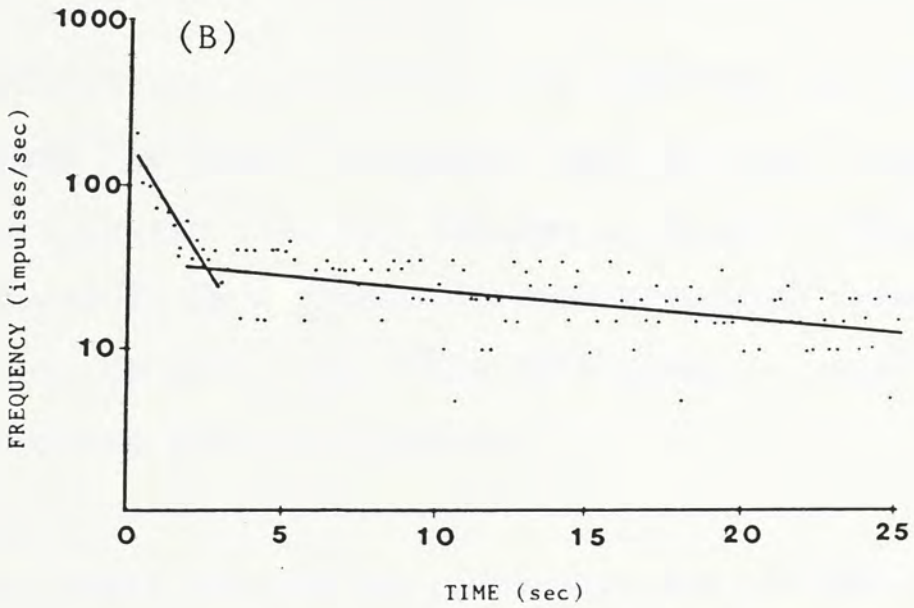


Figure 16A. Discharge of a typical guinea pig SAI unit in response to a sustained constant displacement stimulus (1.5mm) for 25s. The stimulus consists of a 200ms ramp phase at the beginning. The receptor shows a high frequency (200 impulses/s) at the onset of stimulation, followed by a rapid decline in discharge and finally a very low decrease in response. The binwidth is 200ms.

Figure 16B. The frequency in Fig. 16A is transformed to a logarithmic scale indicating 2 apparent phases of adaptation. The 2 phases can be characterized by 2 time constants, 1.9s and 134.5s respectively.

respectively in this particular example. All the units studied showed such a behaviour. The ranges of the first time constants were 0.24s to 3.8s and that of the second time constants are 10.1s to 348.4s, indicating a relatively long second adaptation phase. The first phase usually ended within 5 s.

(c)ISI Distribution and its Statistical Modelling

The variability of the ISI can be observed by plotting the values of the intervals one by one forming a successive spike interval diagram as shown in Figure 17. There seems to be a random distribution with respect to the number of ISI, but this only gives a qualitative picture of the ISI distribution.

A useful index with which to measure the variability of ISI distribution is the coefficient of variation (CV) discussed in Section 1.1.2. The CV of the units studied varied from 0.70 to 1.05. A CV approaching 1 is considered characteristic of an exponential distribution of interval time generated by a simple random process (Poisson process). Attempts were made to fit the distribution quantitatively to Poisson distribution. However, because of the characteristic reduction of very short intervals, making an appearance of a truncated Poisson shape, a more general family of distribution, namely the Gamma distribution, was used to fit the

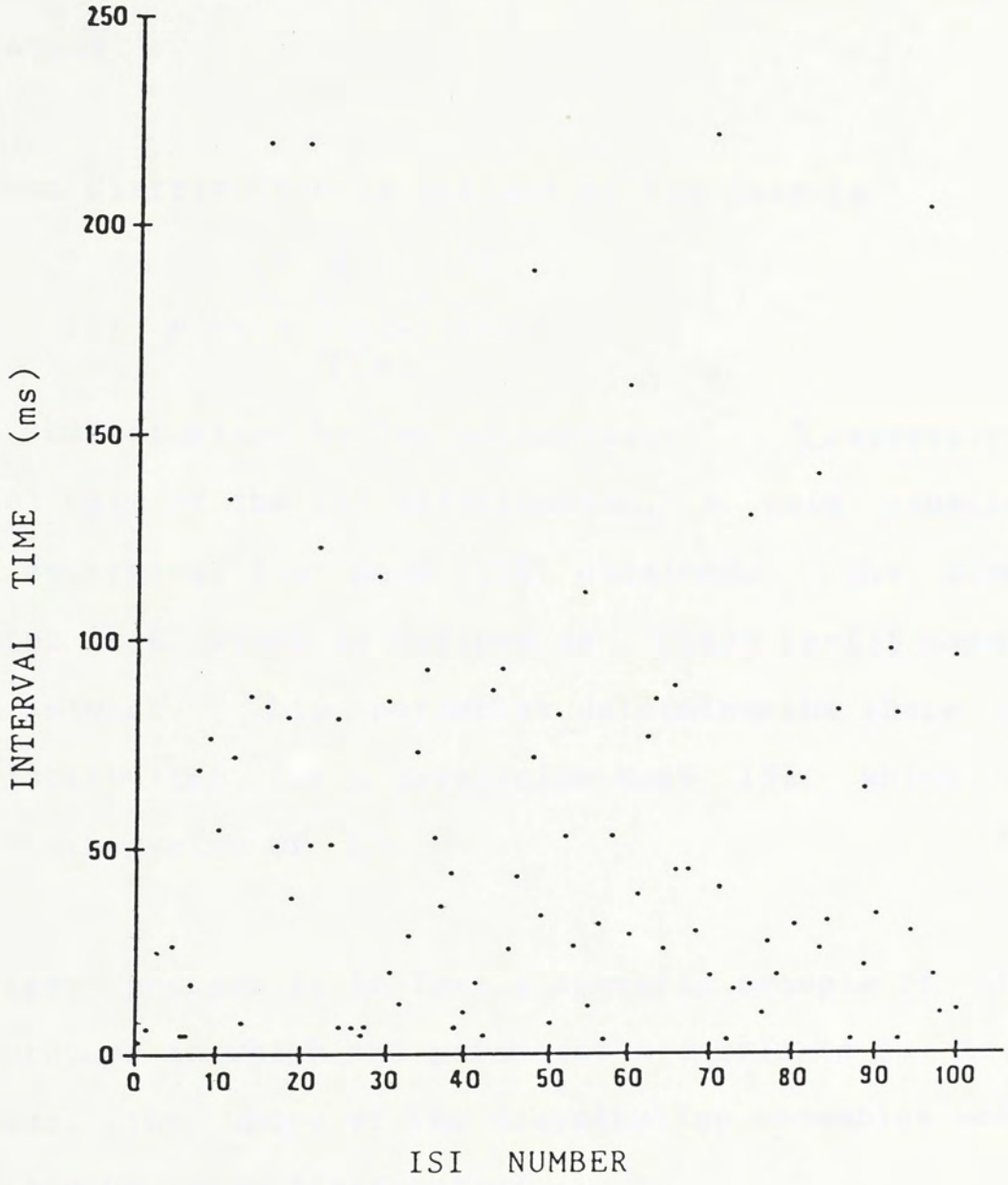


Figure 17. A series of 100 consecutive ISI values obtained from an SAI unit at its fully adapted state and plotted against their number. The values of ISI are randomly distributed.

various ISI distributions of the units under maximal stimulation.

The Gamma distribution is defined by the formula

$$f(t) = \frac{\lambda^k t^{k-1} e^{-\lambda t}}{\Gamma(k)}$$

and is characterized by two parameters: λ represents, in the case of the ISI distribution, a rate constant which determines the mean ISI obtained. The other parameter is k which is defined as $\Gamma(k) = (k-1)!$ when k is an integer. This parameter determines the shape of the distribution for a particular mean ISI, which is given by the value of k/λ.

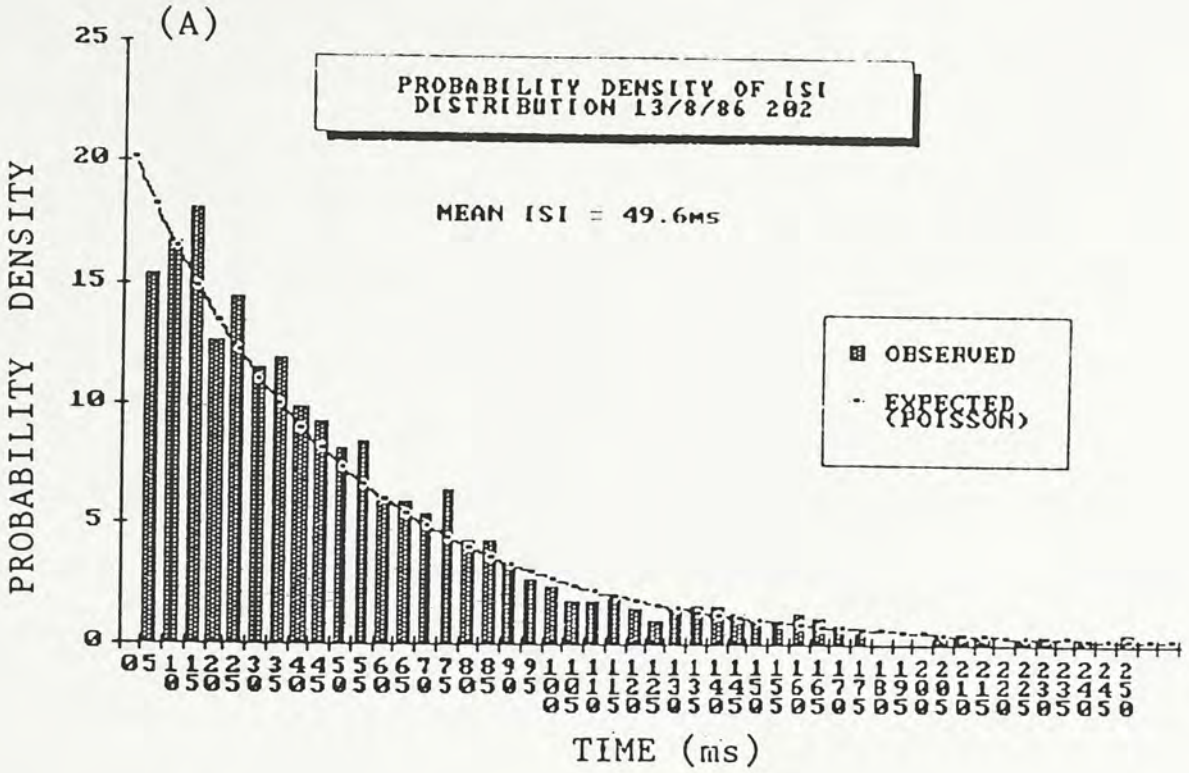
The Poisson process is in fact a specific example of the Gamma process in which the parameter k equals to 1. As k increases, the shape of the distribution resembles more and more a Gaussian distribution.

Apparently the shape of the ISI distribution is affected by the total number of intervals obtained from a particular unit. In order to avoid this, either a percentage frequency or a probability density function can be used. By definition, the probability density (P.D.) function of an interval t is defined as

$$f(t) = \frac{dp(t)}{dt}$$ where p(t) is the probability of interval t

This kind of definition generates a continuous function of the probability density and the probability of a particular interval from t1 to t2 is represented by the area under the curve, between t1 and t2. The total area under the curve, therefore, is equal to one. In practice, the P.D. is calculated by dividing the number of occurrences of a particular interval by the total number of intervals obtained to give the probability. This in turn is divided by the binwidth (in seconds) to generate the P.D. of the interval concerned. A computer program was written to fit the observed ISI distribution of an SAI unit to the theoretical P.D. distribution (generated to fit the same mean ISI and by substituting various integer values of k) by a Chi-square test. The binwidth is limited by the resolution of the sampling system. A resolution of 1 ms was used which was sufficient for most purposes. In the results presented here, a combined binwidth of 5 ms is used.

Most of the ISI distributions could be fitted to a Gamma distribution of parameter k being equal to 1 or 2. For example, Figure 18 shows an ISI distribution of mean value 49.6 ms. The distribution was fitted to the expected exponential distribution (according to the mean ISI value) of P.D. very well, as indicated by a very low value of Chi-square. The distribution, on the other



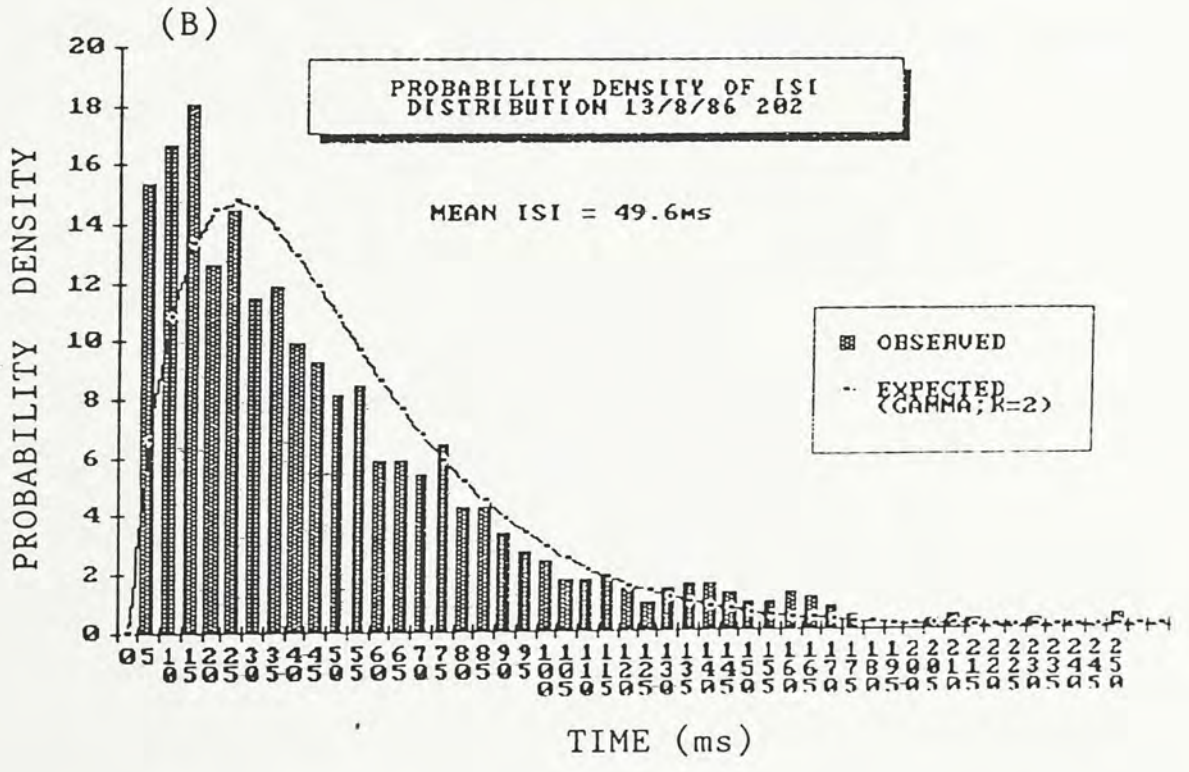


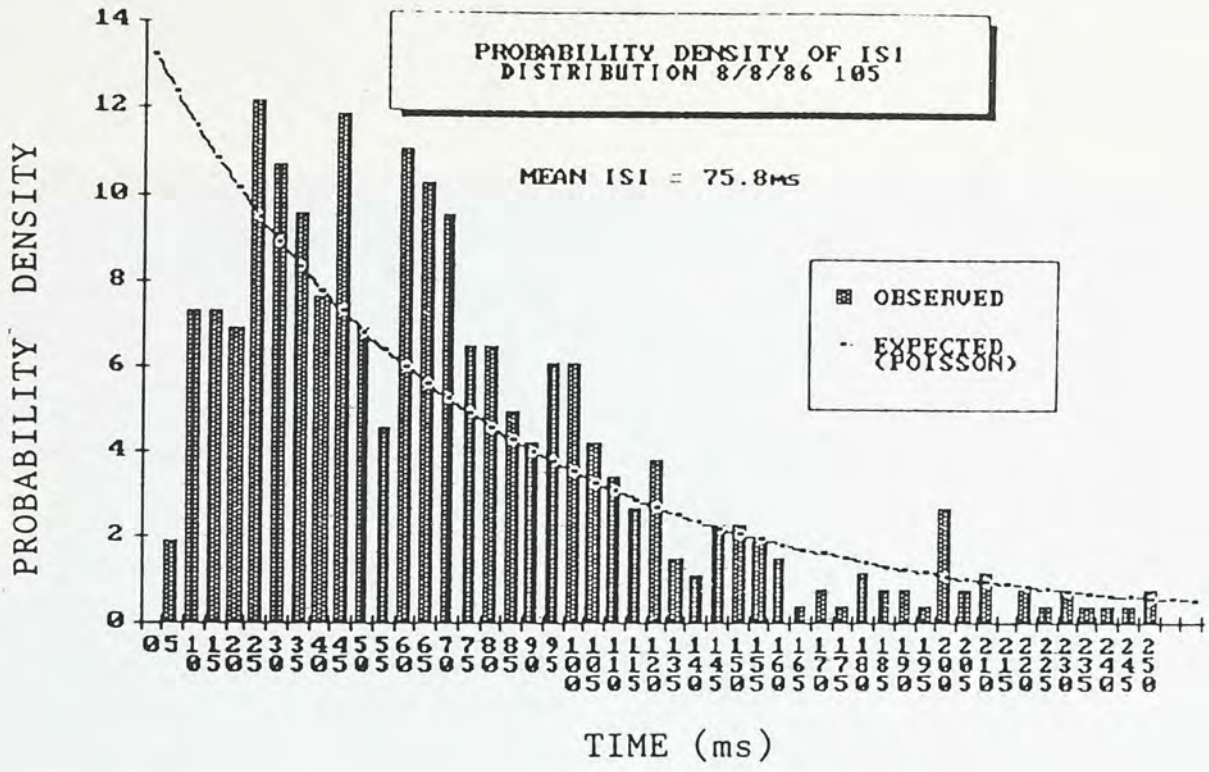
Figure 18. Probability density function of ISI distribution of a fully adapted guinea pig SAI unit fitted by an exponential distribution (or Gamma distribution with shape parameter, k =1). The continuous line in A is the theoretical curve generated when k=1 and taking the mean of the observed distribution, 49.6ms. In B, k=2. In A, χ^2-test shows a goodness of fit, but not in the case of B. Total number of intervals obtained is 1245. Mean ISI=49.6ms.

hand, could not be fitted to a Gamma distribution of k=2 by the Chi-square test.

Figure 19(a) and (b) show another example of ISI distribution that could not be fitted to the Poisson distribution but which has a good fit to a Gamma distribution of k=2. Summing up, there were 6 units that could be described by Poisson and 5 units by a Gamma distribution of k=2. There was one ISI distribution that could not be fitted by values of 1 or 2 or other integers. In the event that both k=1 and k=2 showed a significant fit ($p<0.05$), the one yielding the smaller Chi-square value was accepted.

3.1.2 Ultrastructure

The presence of Merkel cell-neurite complexes was confirmed by the use of electronmicroscopy. Figure 20 shows a Merkel cell and its relationship with the terminal neurites. The most characteristic feature of the cell was the presence of round electron-dense granules (g), located mainly on the area apposing the neurite. These granules allowed the Merkel cell to be distinguished with certainty from other non-keratinocytes. Mitochondria (m) and other larger electron-dense granules could also be seen in the cytoplasm. The nucleus was conspicuous with irregular shape. Desmosomes could not be located in the



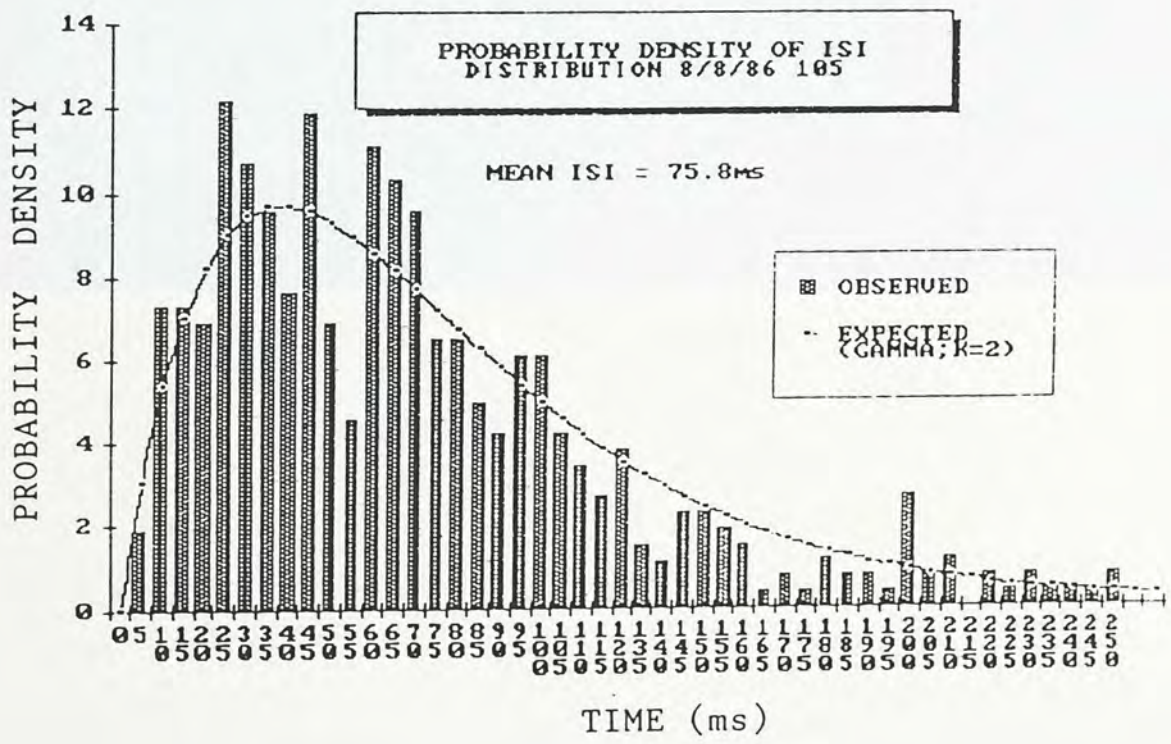


Figure 19. Probability density function of ISI distribution of another fully adapted guinea pig SAI unit with mean=75.8ms. The distribution is fitted better by a Gamma distribution with k=2 then a Poisson distribution with k=1.

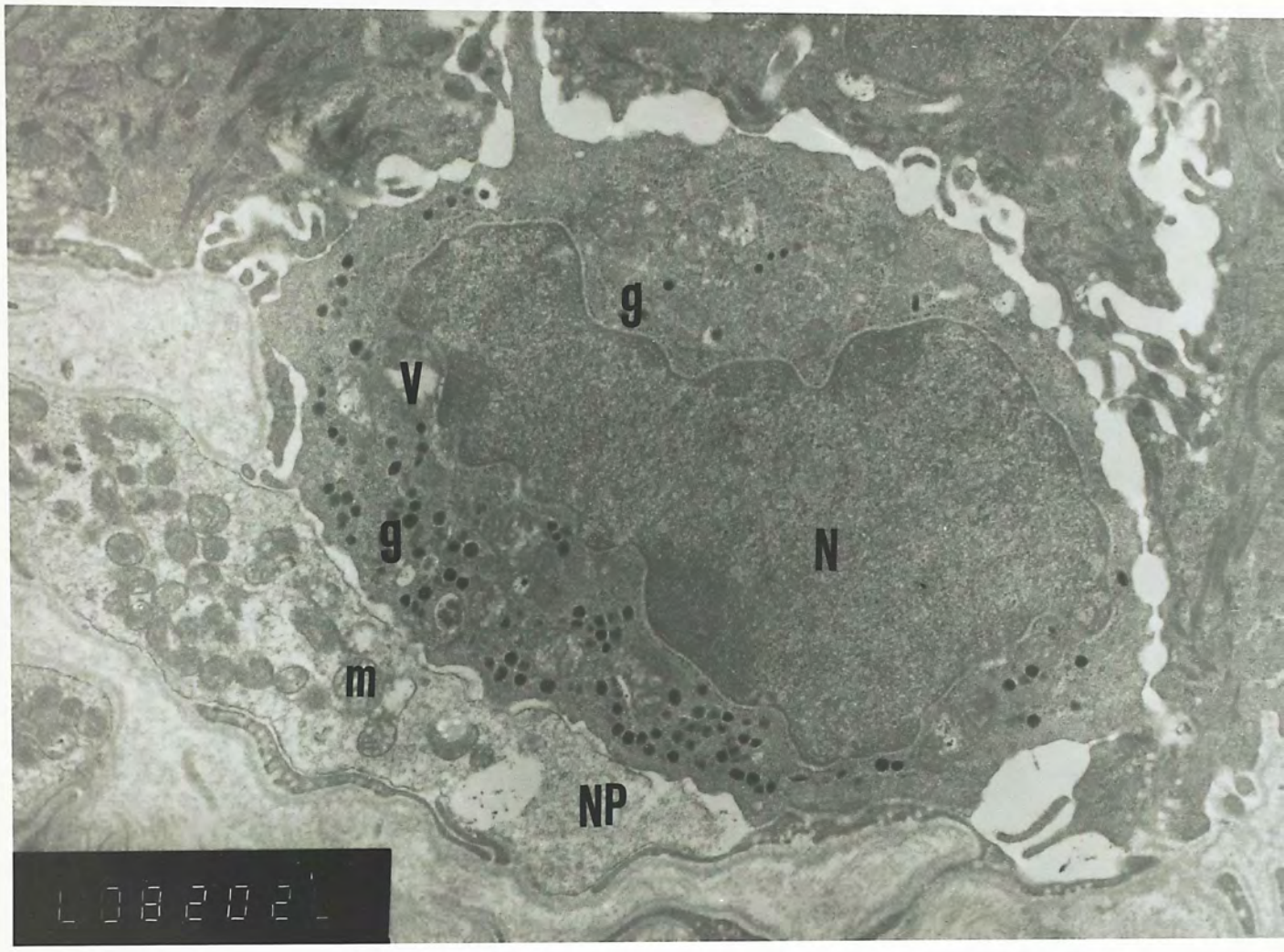


Figure 20. A Merkel cell and associated nerve plate (NP). The Merkel cell shows typical conspicuous irregularly-shaped nucleus (N) and dark-cored granules(g). Some empty vesicles (V) are found. Note that most of the granules occur in the area apposing the nerve plate. Numerous mitochondria(m) are found in the nerve plate. x14400.

specimens but there existed typical finger-like projections from the epidermal side of the Merkel cells. Some vesicles (V) which were not membrane-limited could be found in the cytoplasm. These might represent globules of lipid or some other substance lying free in the cytoplasm and lost during processing (Breathnach, 1971b). The micrograph also showed the close association of the Merkel cells with a terminal neurite on the dermal side and which was characterized by the presence of a large number of mitochondria.

Figure 21 illustrates that between Merkel cell and the nerve terminal there were various places of particular association, which were similar to those described in Merkel cells of other mammals (Chen et al., 1973; Cheng-Chew et al., 1984).

Figure 22 is a highly magnified (x38000) picture of the granules. The dense-cored granules seemed to be membrane-limited with a core of variable density.

3.2 EFFECTS OF ACUTE ADMINISTRATION OF NEOMYCIN IN GUINEA PIG

3.2.1 Compliance of the skin

In order to maintain the constant force of 20mN, the

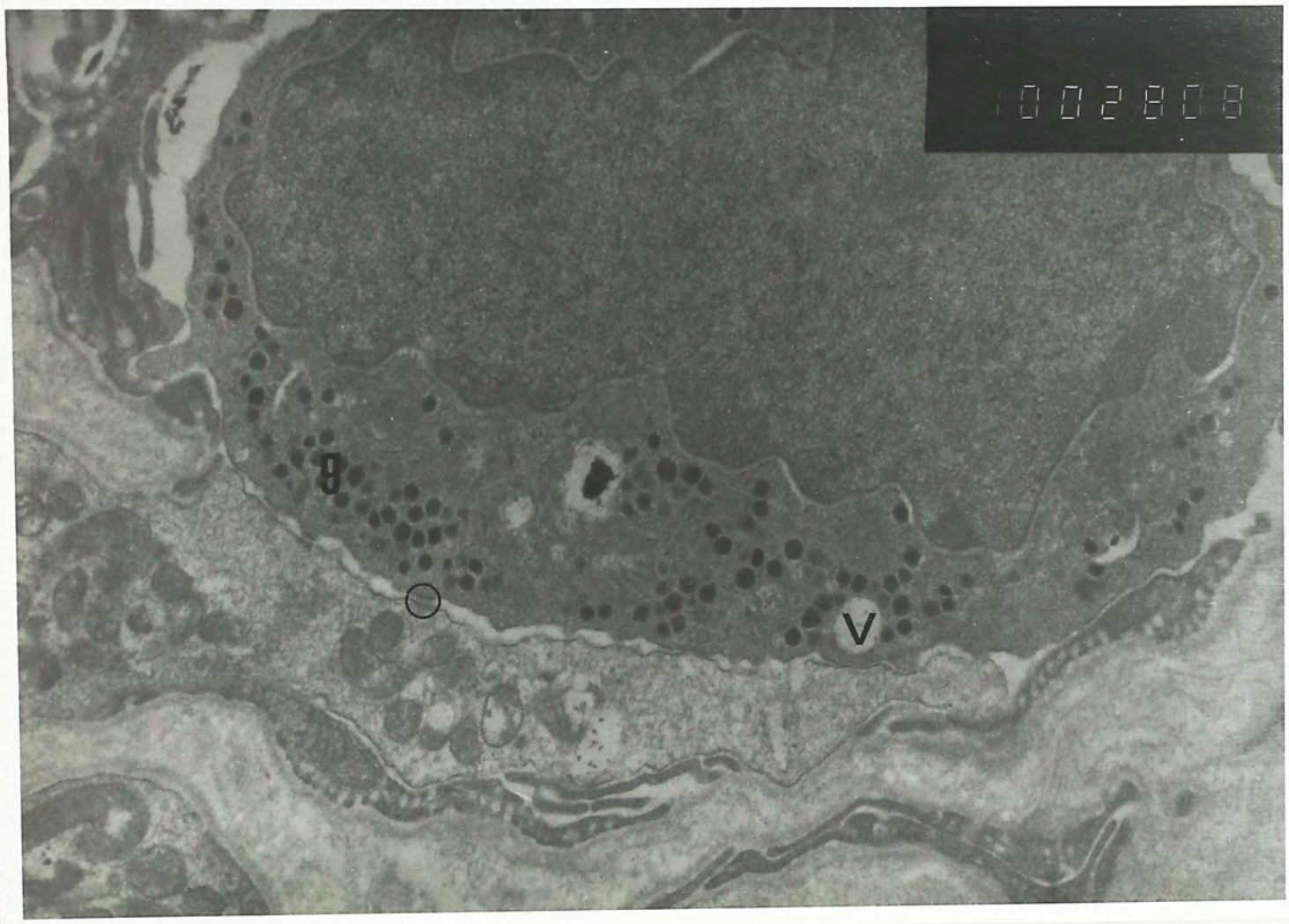


Figure 21. A Merkel cell-neurite complex in higher magnification, showing some vesicles(V) which are not membrane-bound and dark cored granules (g). The circle shows an area of thickening of the plasma membrane in both Merkel cell and apposed neurite. x 20000.

Figure 22. A highly magnified picture of the dense -cored granules (g) showing their various sizes and variable density. x38000

average values (n=8) of residual indentation, maximal indentation and stroke amplitude (maximum indentation - residual indentation) were 0.05±0.02mm, 0.91±0.12mm, and 0.86±0.11mm respectively during the control period of saline infusion. After the infusion of neomycin, there were no significant changes in the values of the parameters measured. They were 0.06mm±0.02mm, 0.97±0.14mm and 0.91±0.12mm respectively.

3.2.2 Nervous Response

Figure 23 shows a typical response, during and after the infusion of neomycin of dose 15mg. In the control period, there was an average of about 145 impulses. The infusion started at minute 30 and the nervous response started to decline at minute 40 and was maintained at a mean accumulated impulse count of 40 for a long period without significant recovery.

In order to test whether the neomycin effect was dose-dependent, 3 different doses were tested: 9mg, 15mg and 45mg. The degree of suppression of the neomycin on the dynamic, static and total responses of SAI receptor is summarized in Figure 24. Figure 24(a) shows the percentage of the total response left after different dosages of neomycin had been administered. 80.7%, 48.1% and 34.6% of the control response was left after infusion of 9, 15 and 45mg of neomycin. The percentage suppression

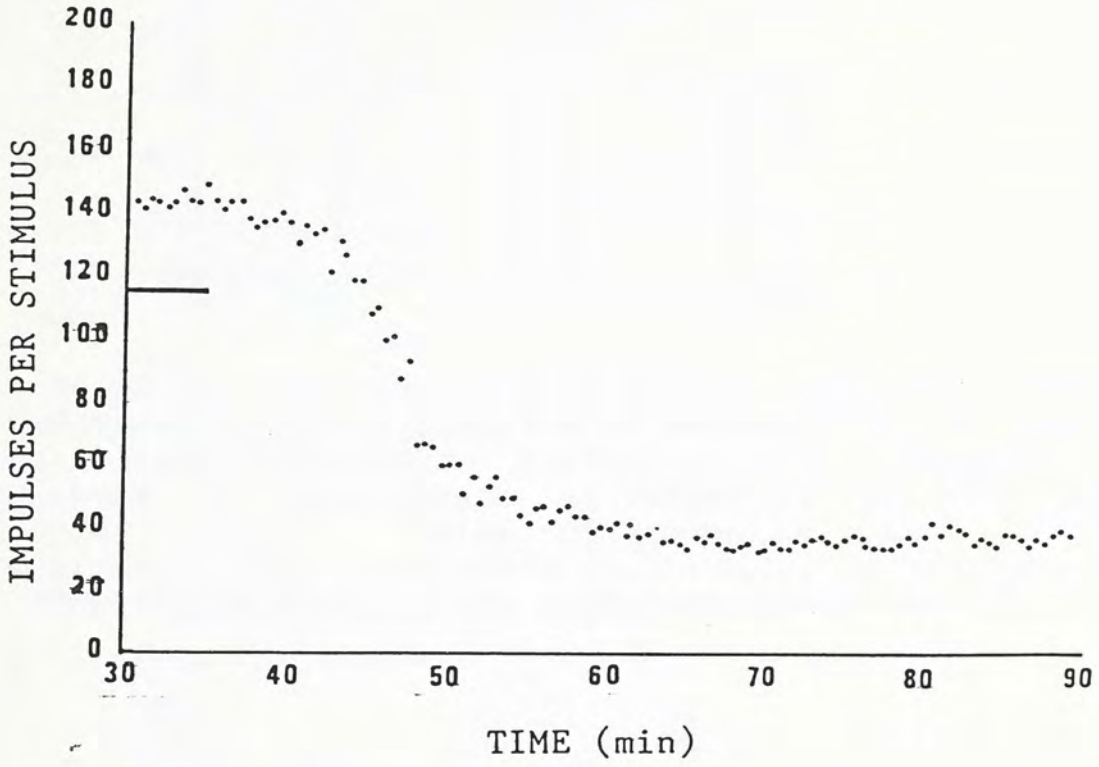


Figure 23. Response of an SAI receptor of guinea pig to standard stimuli of 20mN force applied every 30s. The stimulus was comprised a 200ms ramp phase and 2s plateau phase. From minute 0 to minute 30 (not shown), the total number of spikes per stimulus per stimulus were maintained at about 140. Infusion of neomycin into the limb circulation (total of 15mg) started at minute 30 to minute 35 at a rate of 3mg/min. The latency of the infusion system was 8 minutes.

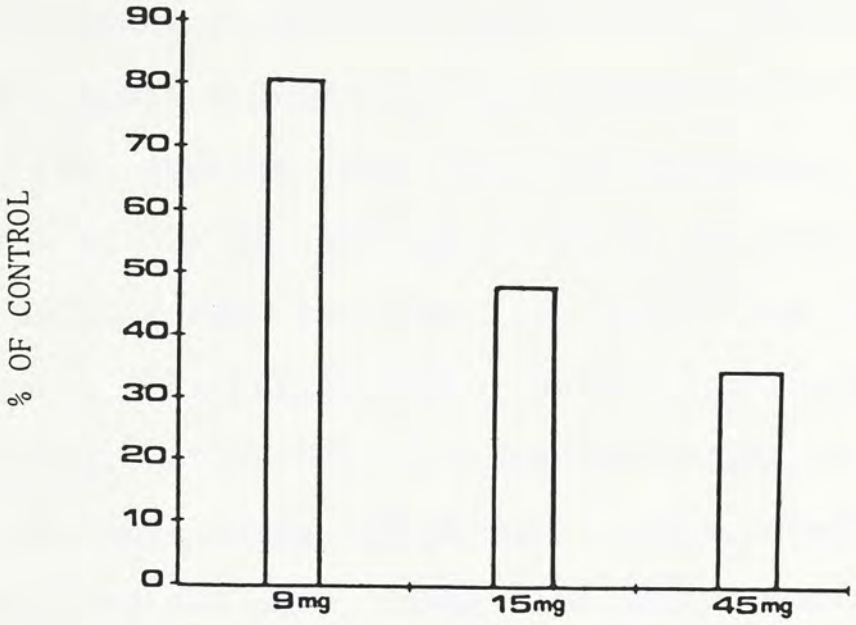


Figure 24A. The responses of guinea pig SAI receptors after close arterial infusion of neomycin (9, 15 and 45mg) into limb circulation perfusing the receptors and expressed as percentages of responses in the control periods before infusion of neomycin. Each stimulus consists of 200ms ramp phase followed by 2s constant force of 20mN and applied to the receptors every 30 s.

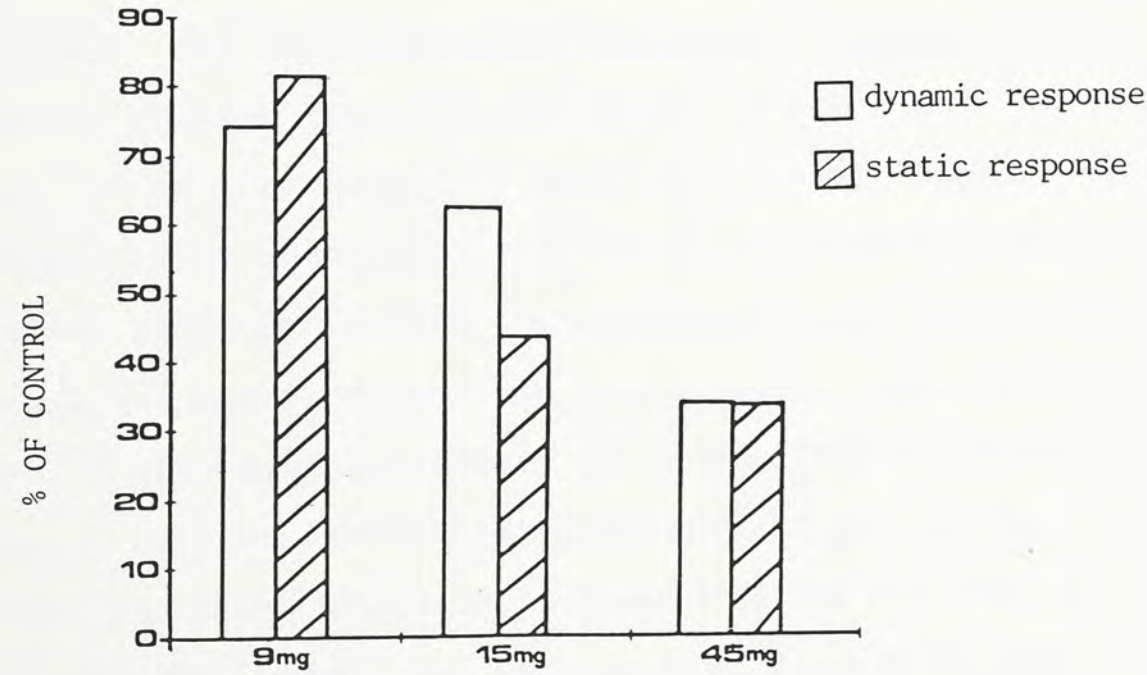


Figure 24B. The dynamic and static responses of guinea pig SAI receptors after close arterial infusion of neomycin (9, 15 and 45mg) and expressed as percentages of response in the control periods before the infusion of neomycin.

thus depended on the dose administered. The data in Fig. 24(b) suggest a dose-dependent inhibition of neomycin on both the dynamic and static responses. For dynamic responses, 74.5%, 63% and 34.6% of the control response was left after infusion of 9mg, 15mg and 45mg of neomycin. The corresponding values for static responses were 82%, 44% and 34.3% respectively. At the dosage of 45mg the percentage inhibition in both dynamic and static phases were the same, namely 65% inhibition.

3.2.3 ISI Distribution

Figure 25 shows the ISI histogram of the control response and treated response of a typical SAI receptor before and after 9mg of neomycin infusion. Qualitatively, neomycin shifted the ISI histogram to the right as a result of a decrease in the total number of impulses. The characteristic of a single mode with a positive skewness of ISI distribution was maintained although the mode shifted from 5ms to 6ms. Figure 26 and Figure 27 show similar effects of 15 and 45mg of neomycin infusion on two other SAI receptors. After 15 mg in another SAI unit, the mode shifted from 6ms to 7ms and after 45mg in another unit, the mode shifted from 12ms to 22ms. The cumulative percentage incidence had also been plotted for the responses under the 3 dosages, as shown in Figure 28. Quantitatively there were significant

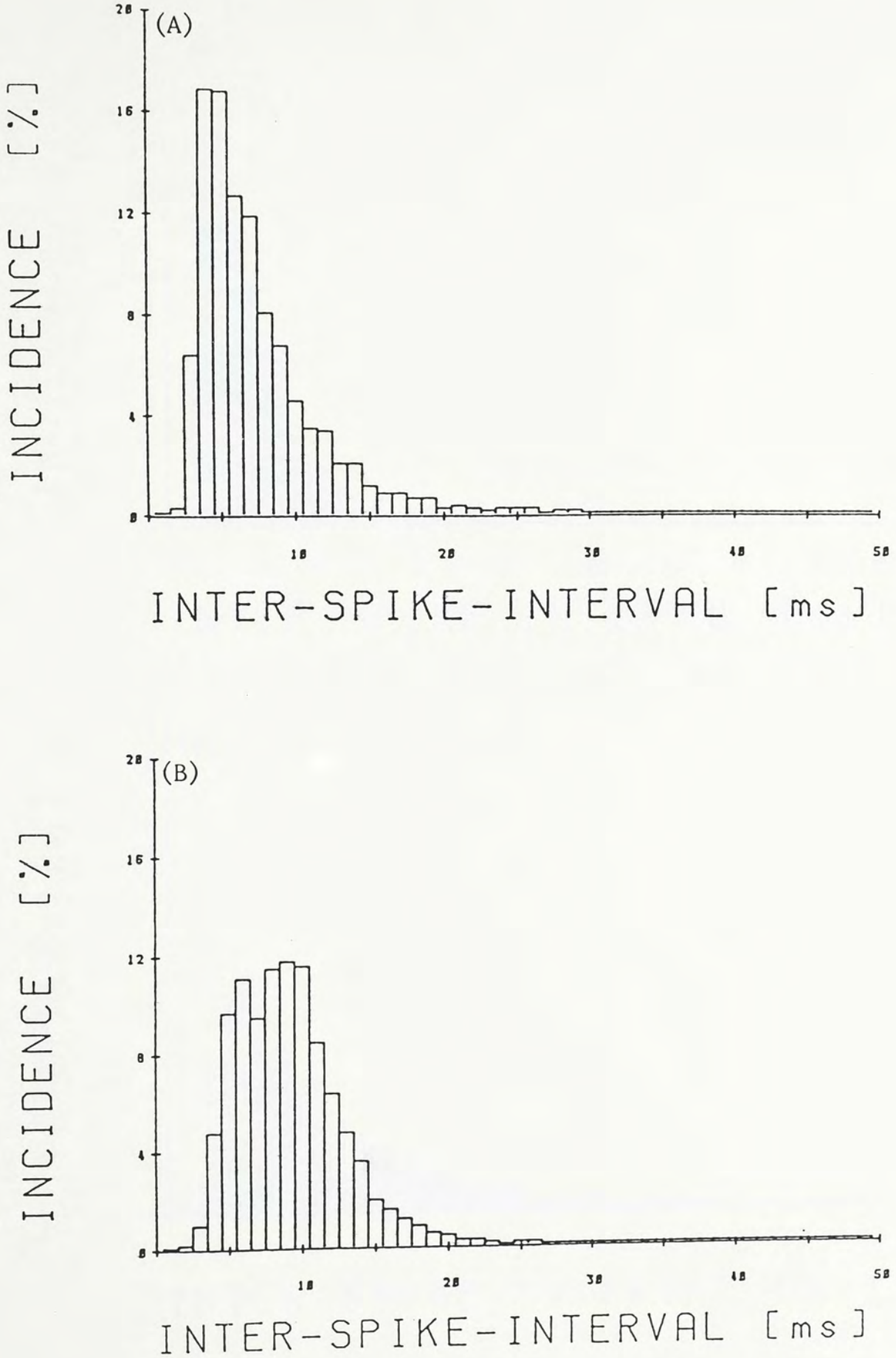


Figure 25. The percentage distribution of ISI of a typical guinea pig SAI receptor during full period (0-2200ms) of responses. Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 9 mg of neomycin.

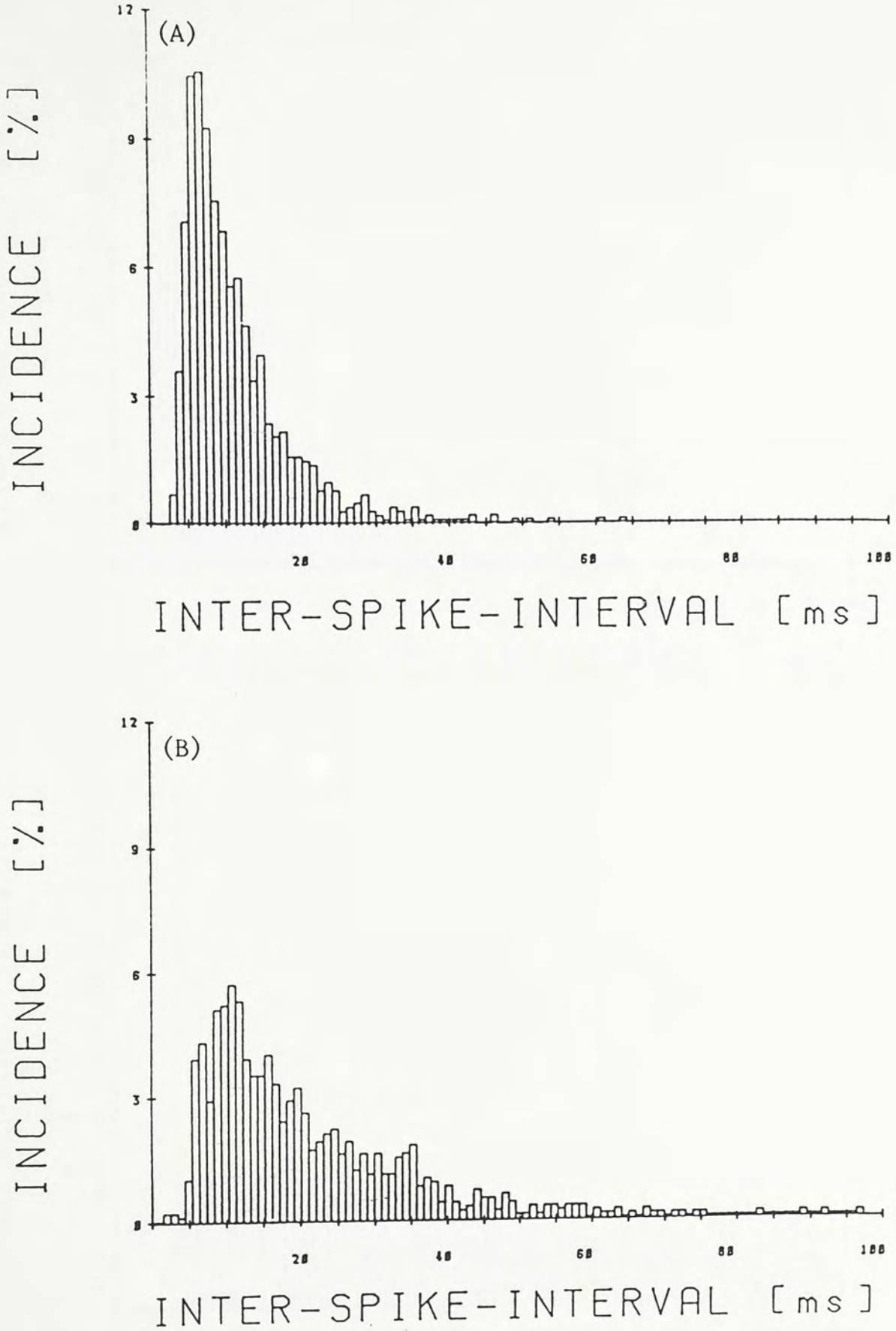


Figure 26. The percentage distribution of ISI of a typical guinea pig SAI receptor during full period (0-2200ms) of responses. Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 15mg of neomycin.

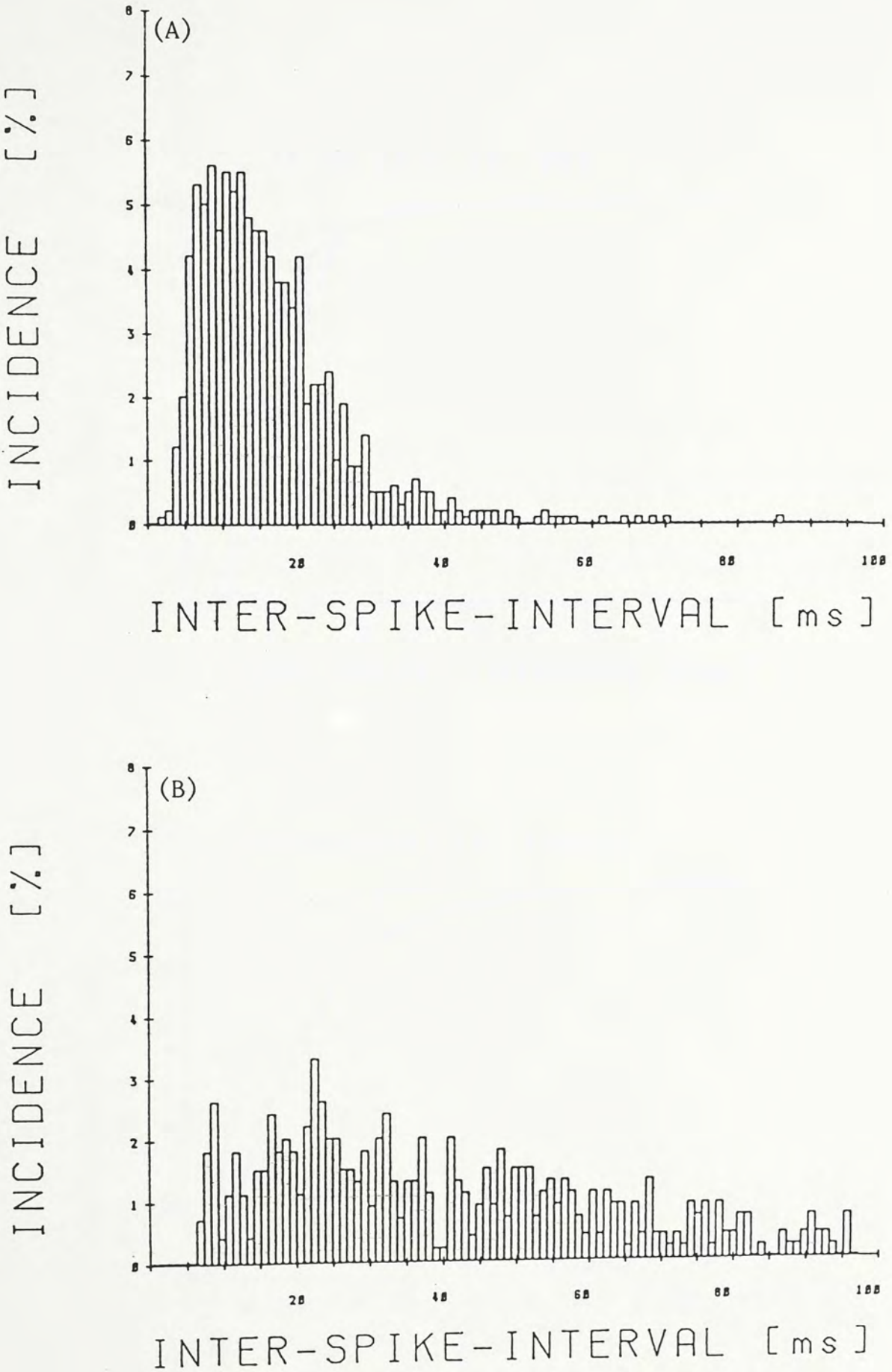
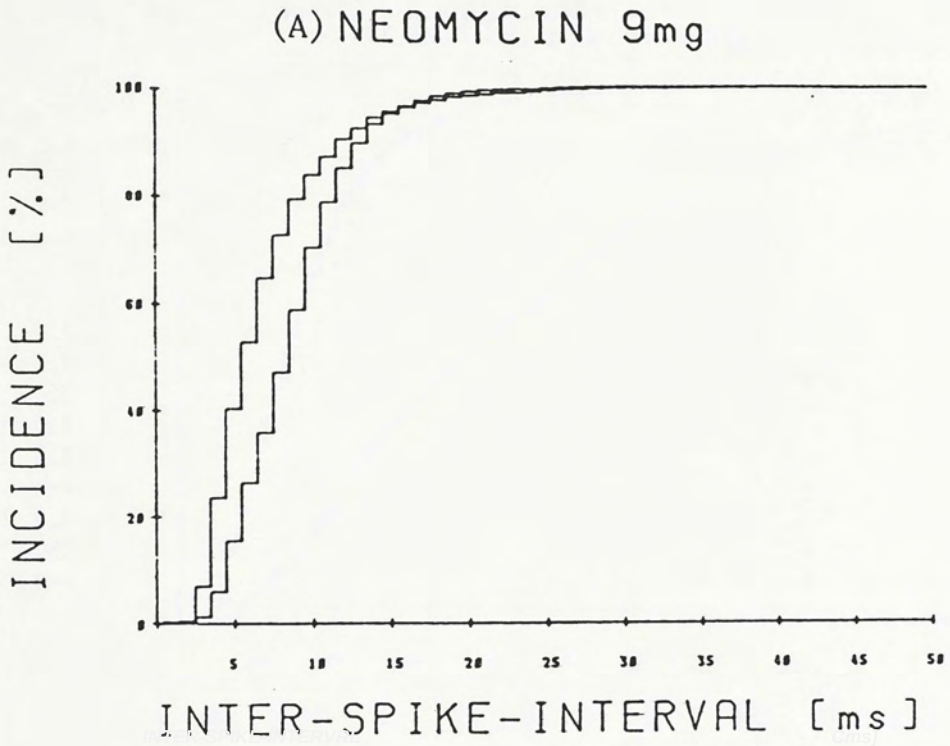
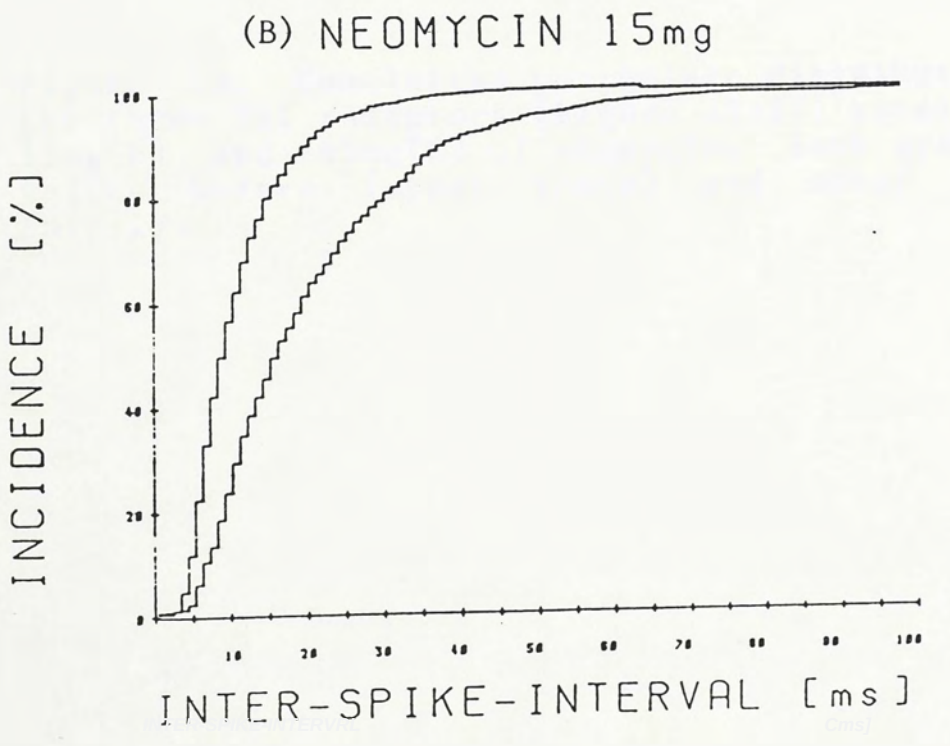


Figure 27. The percentage distribution of ISI of a typical guinea pig SAI receptor during full period (0-2200ms) of responses. Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 45mg of neomycin.


(A) NEOMYCIN 9mg
INCIDENCE [%]
INTER-SPIKE-INTERVAL [ms]


(B) NEOMYCIN 15mg
INCIDENCE [%]
INTER-SPIKE-INTERVAL [ms]

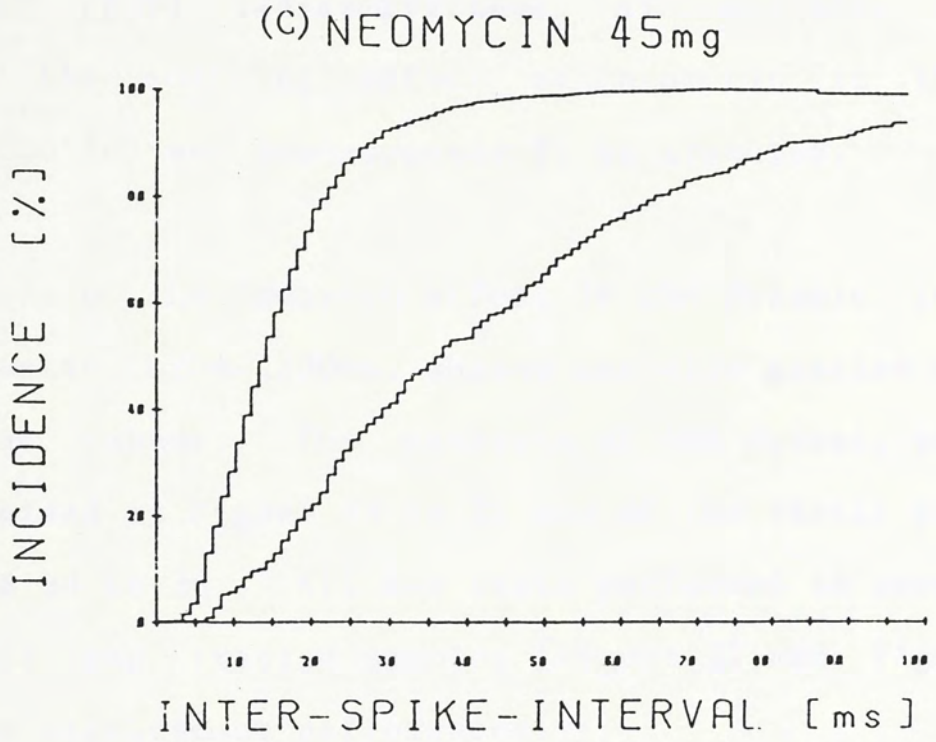


Figure 28. Cumulative percentage distribution of ISI of the three SAI receptors (Figure 25-27) receiving 9mg (A), 15mg(B) and 45mg(C) of neomycin. Each graph shows the values before (upper trace) and after (lower trace) infusion.

changes in all the samples compared by a Kolmogorov-Smirnov (K-S) two-sample test. It could also be seen that the shifting effect of neomycin in the ISI distribution was dose-dependent, as expected.

Analysis of the neomycin effect in the dynamic (0-250ms) and static (1200-2200ms) phases was also carried out in a similar manner. The analysis of the dynamic phase is summarized in Figure 29 to 32 and of the static phase in Figure 33 to 36. All K-S tests performed to compare the control and treated samples (Figure 32 and Figure 36) showed significant differences.

3.3 EFFECTS OF CHRONIC ADMINISTRATION OF LITHIUM IN RATS

3.3.1 By Food

(a)General condition of the rat

The body weight, food and water consumptions of both the control and treated rats were recorded every day to detect possible symptoms of intoxication early. It was found that while the water consumption of treated rats increased gradually with days of treatment, the body weights of the two groups were not significantly different. There was also no difference in the food consumption. The average food consumption was 90g/kg body weight and therefore the dosage administered was

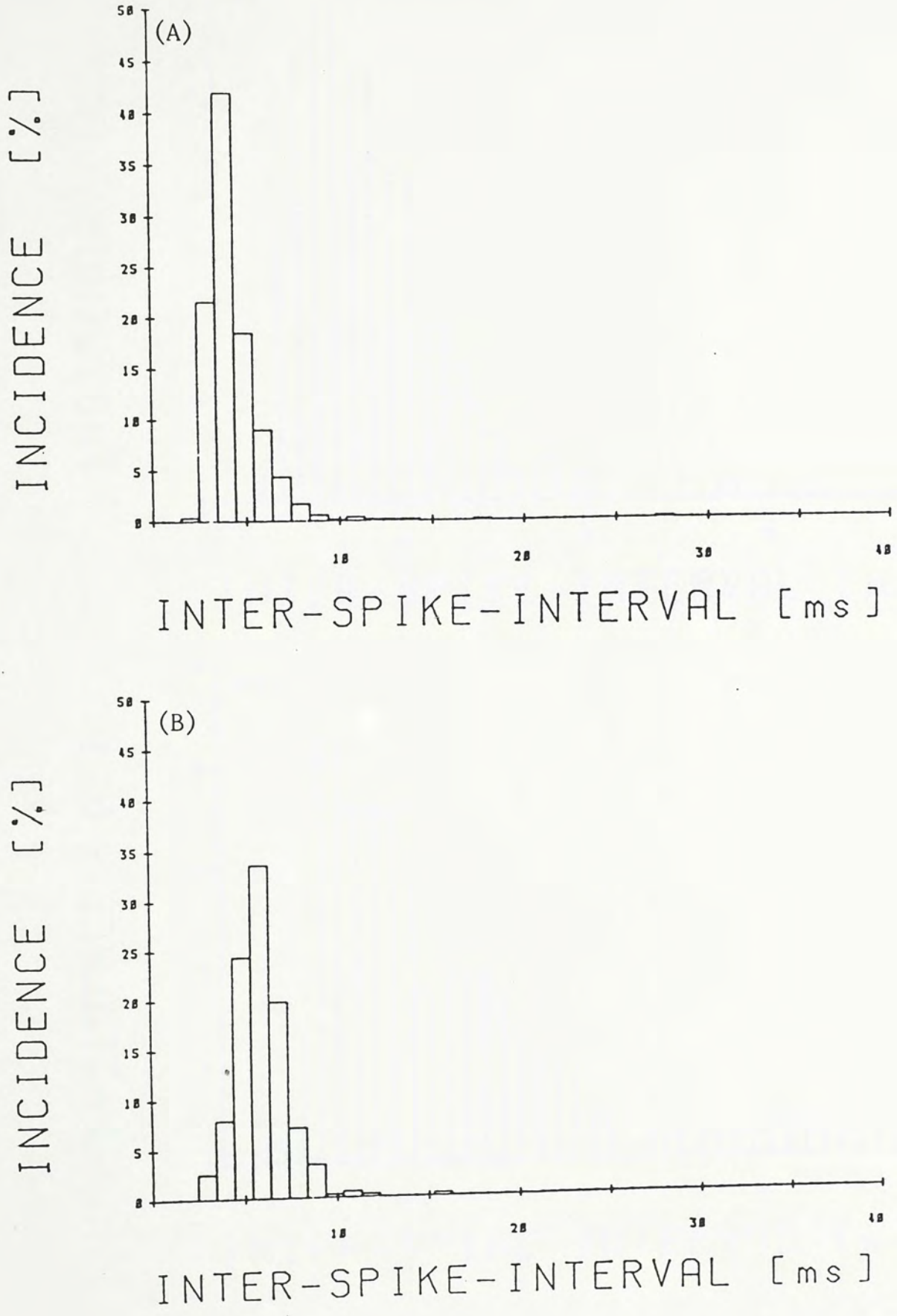


Figure 29. The percentage distribution of ISI of a typical guinea pig SAI receptor during dynamic response (0-250ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 9 mg of neomycin.

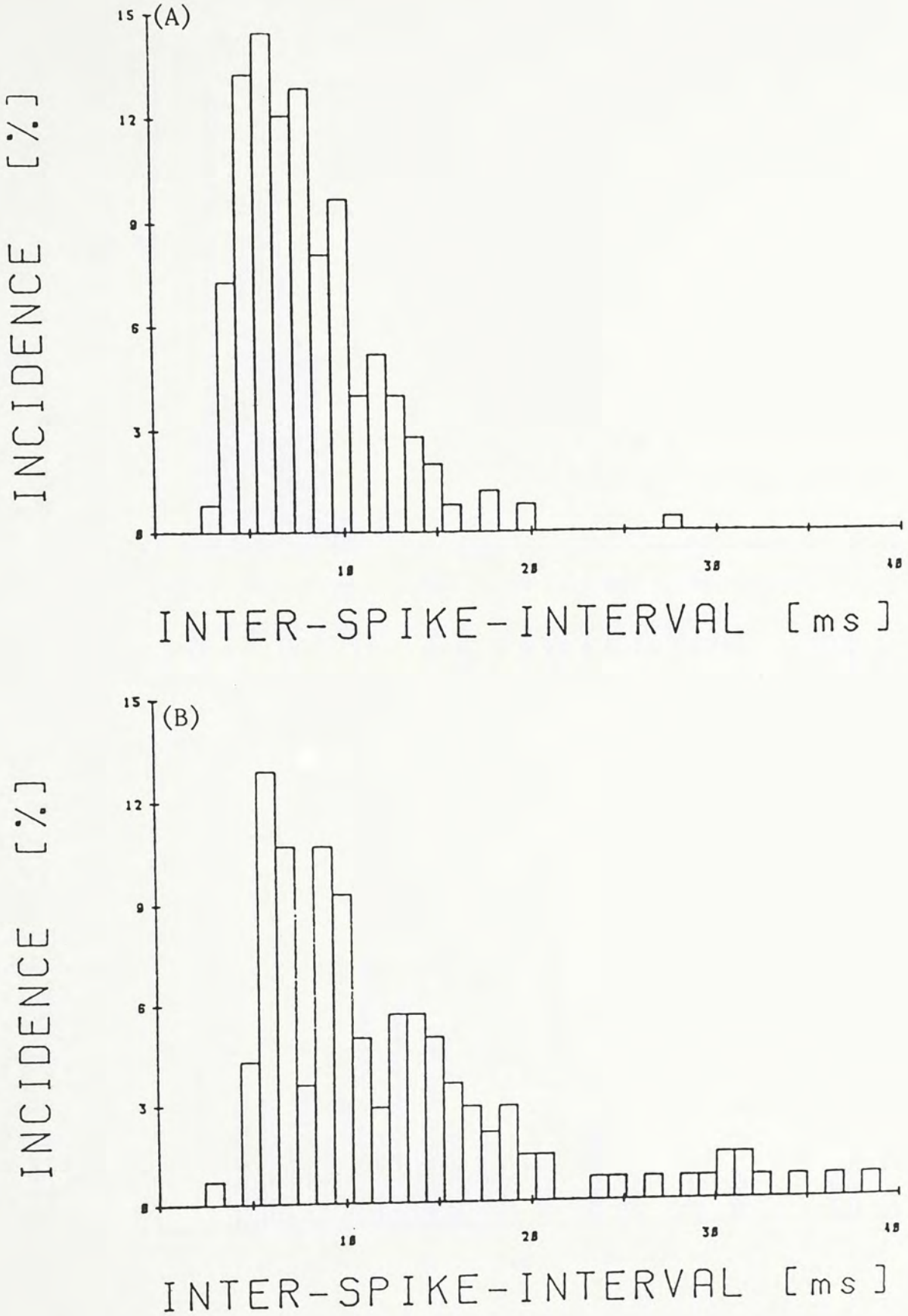


Figure 30. The percentage distribution of ISI of a typical guinea pig SAI receptor during dynamic response (0-250ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 15mg of neomycin.

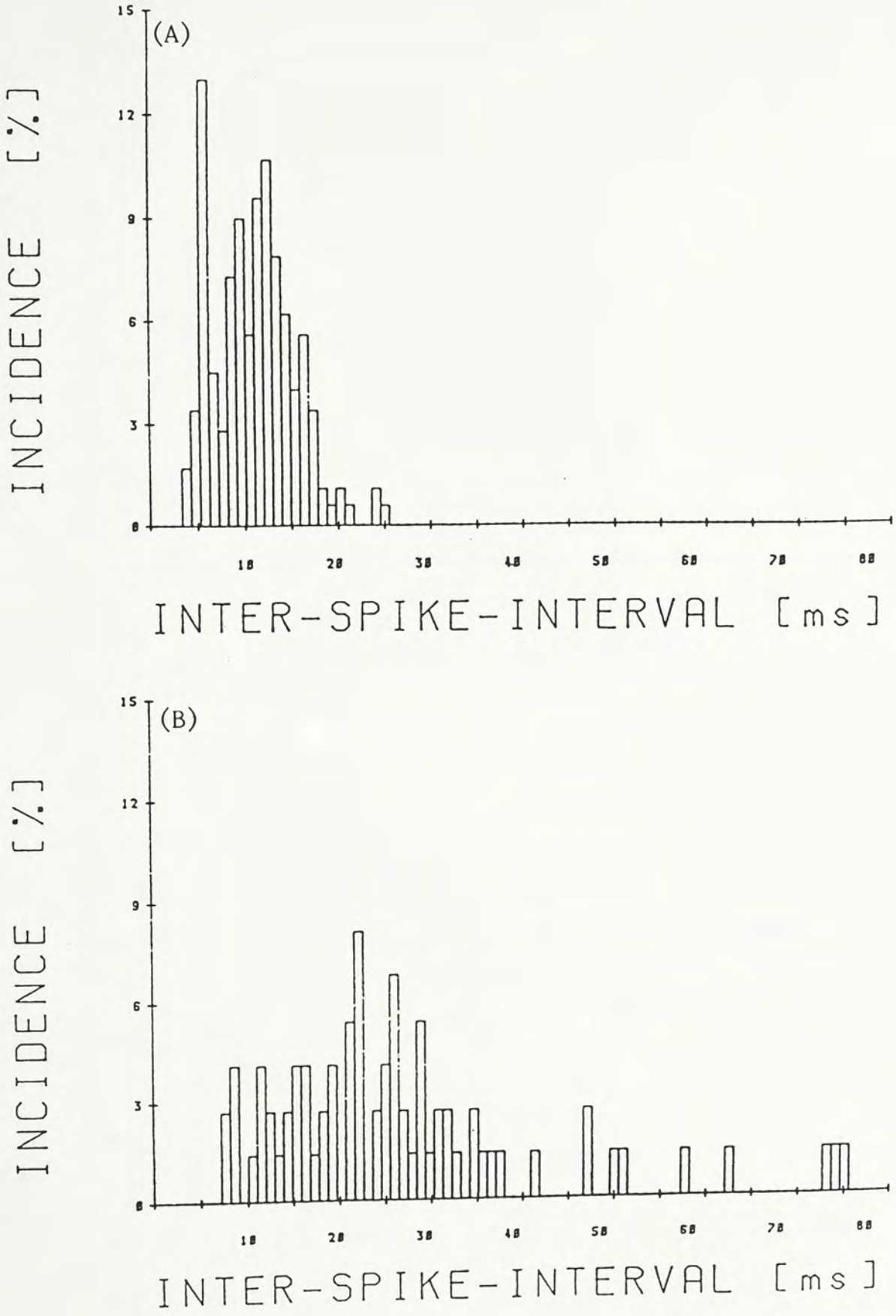
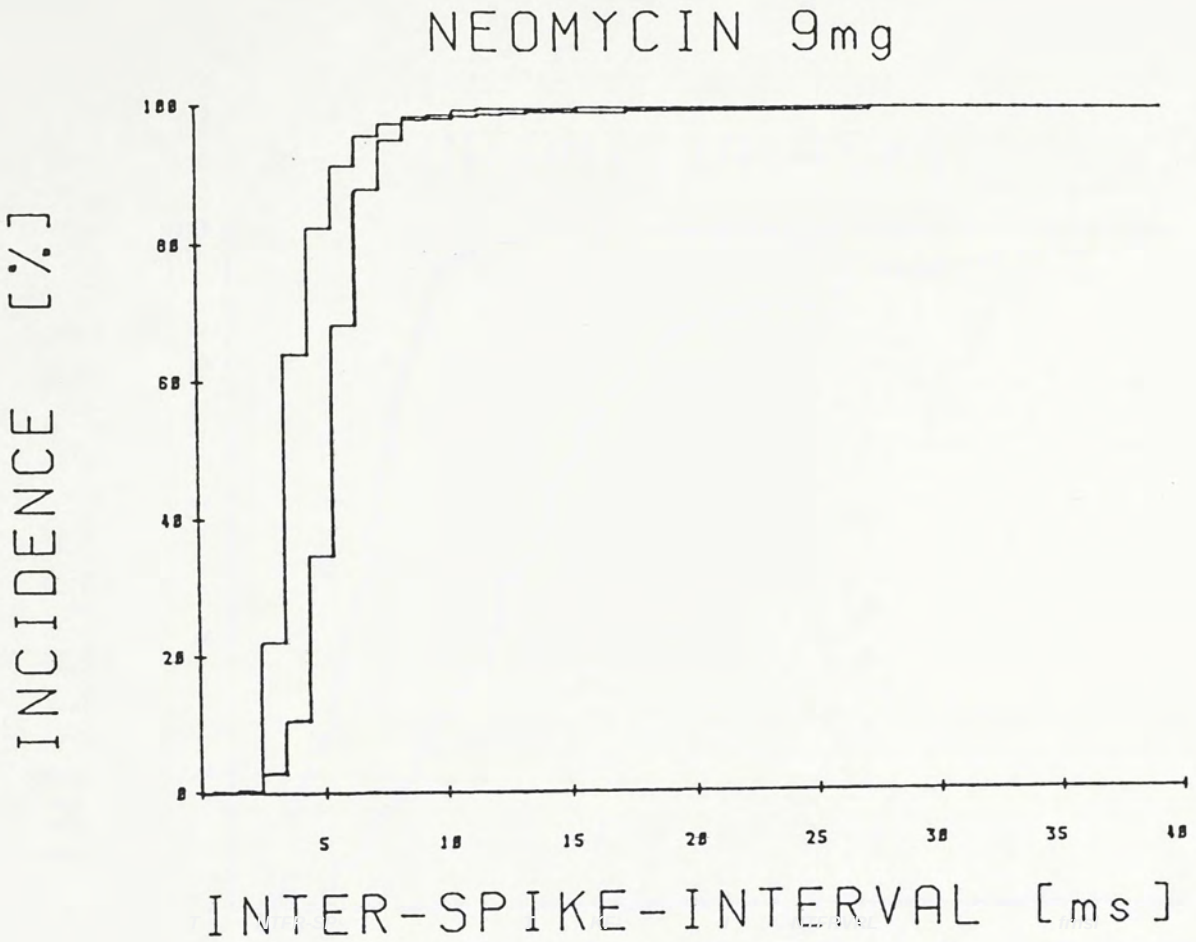
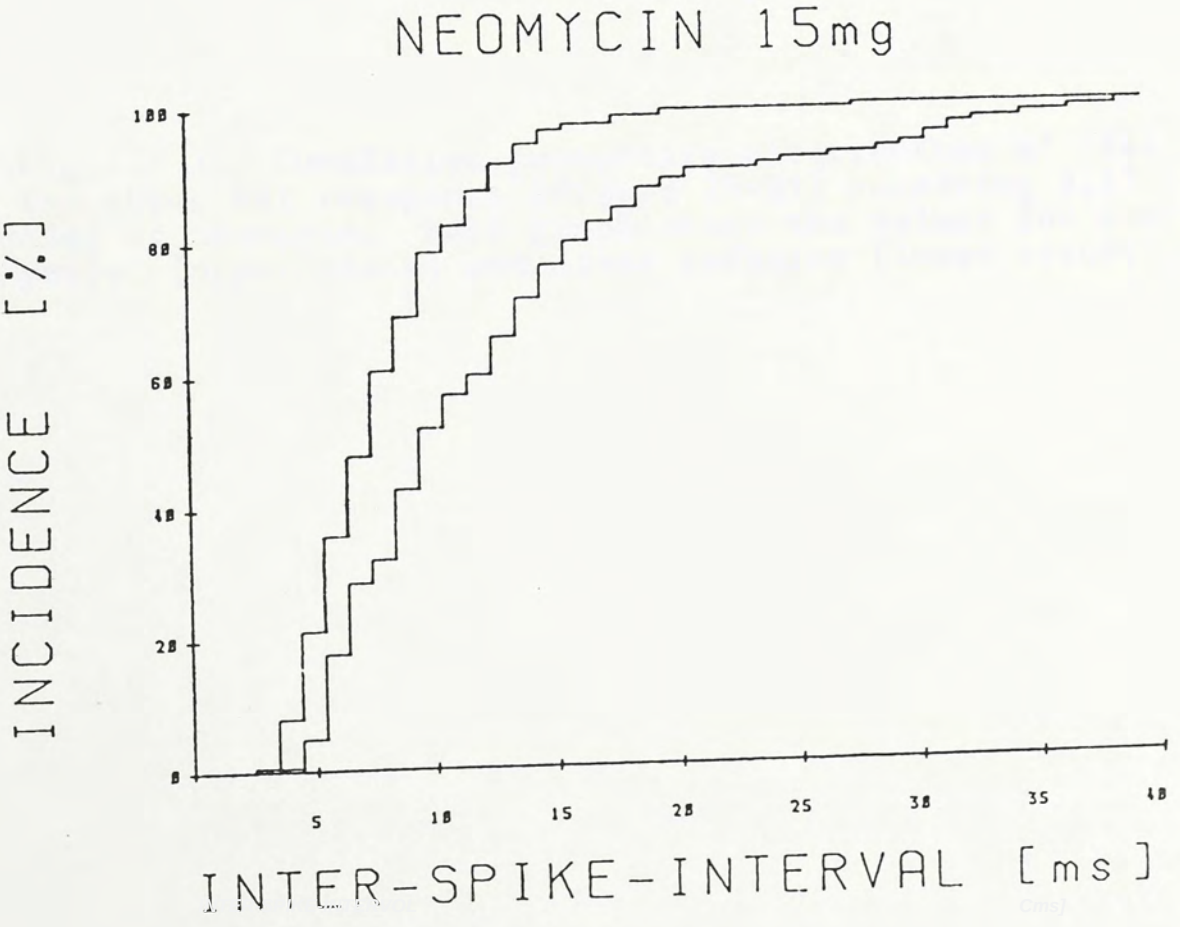


Figure 31. The percentage distribution of ISI of a typical guinea pig SAI receptor during dynamic response (0-250ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 45mg of neomycin.


NEOMYCIN 9mg
INCIDENCE [%]
100
80
60
40
20
0
5
10
15
20
25
30
35
40
INTER-SPIKE-INTERVAL [ms]


NEOMYCIN 15mg
INCIDENCE [%]
100
80
60
40
20
0
5
10
15
20
25
30
35
40
INTER-SPIKE-INTERVAL [ms]

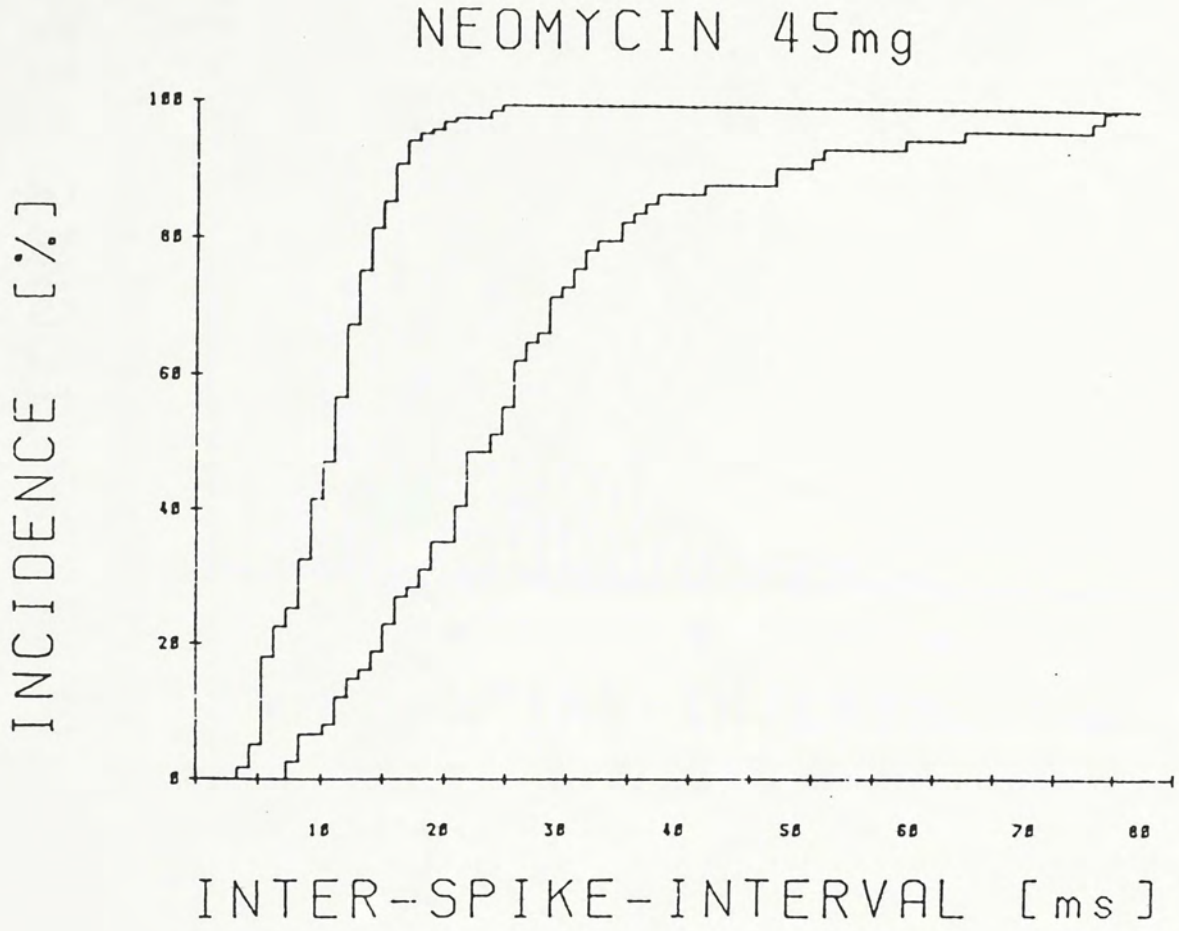


Figure 32. Cumulative percentage distribution of ISI of the three SAI receptors (Figure 29-31) receiving 9,15 and 45mg of neomycin. Each graph shows the values for control period (upper trace) and after infusion (lower trace).

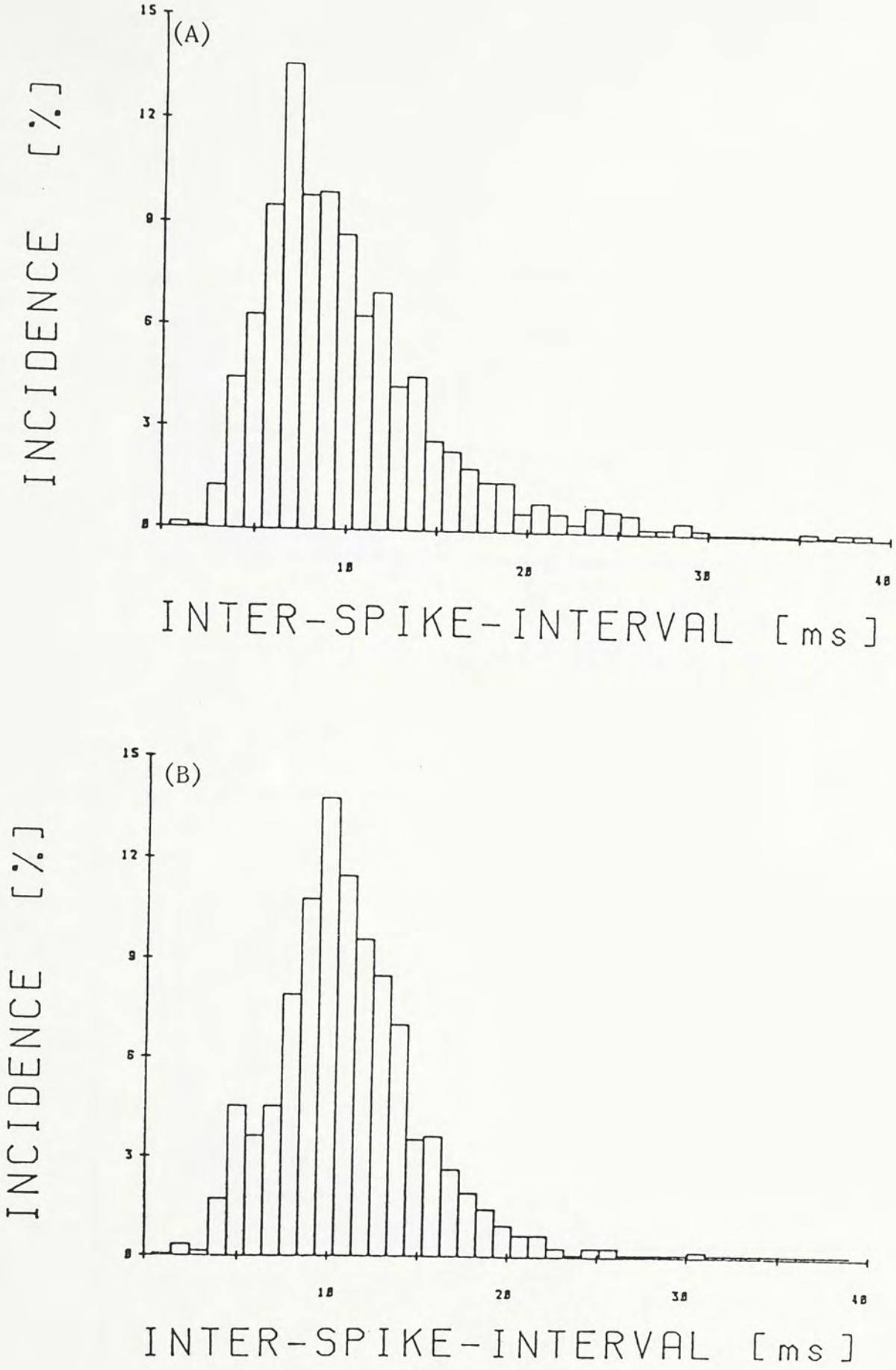


Figure 33. The percentage distribution of ISI of a typical guinea pig SAI receptor during static response (1200-2200ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 9 mg of neomycin.

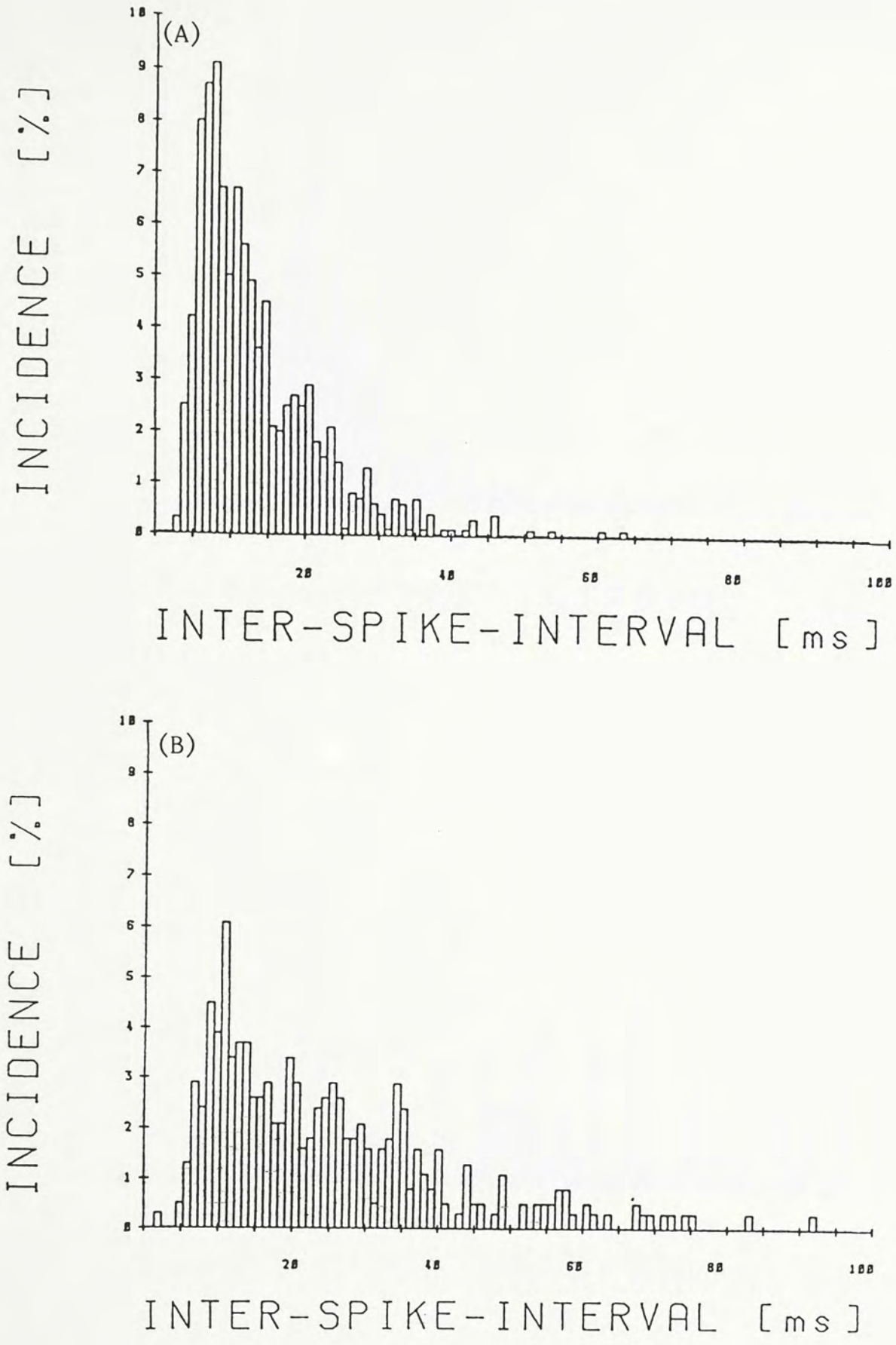


Figure 34. The percentage distribution of ISI of a typical guinea pig SAI receptor during static response (1200-2200 ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 15 mg of neomycin.

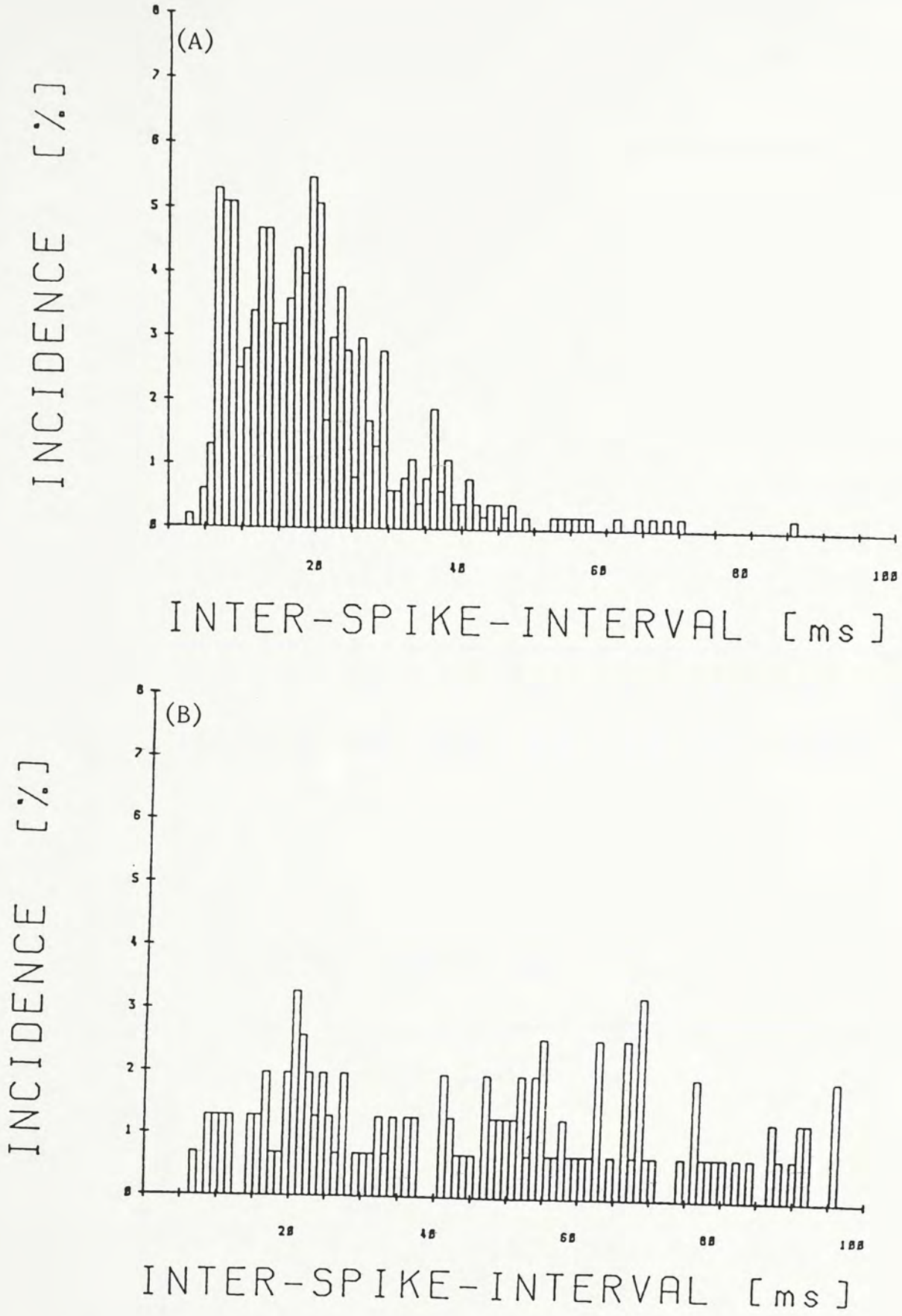
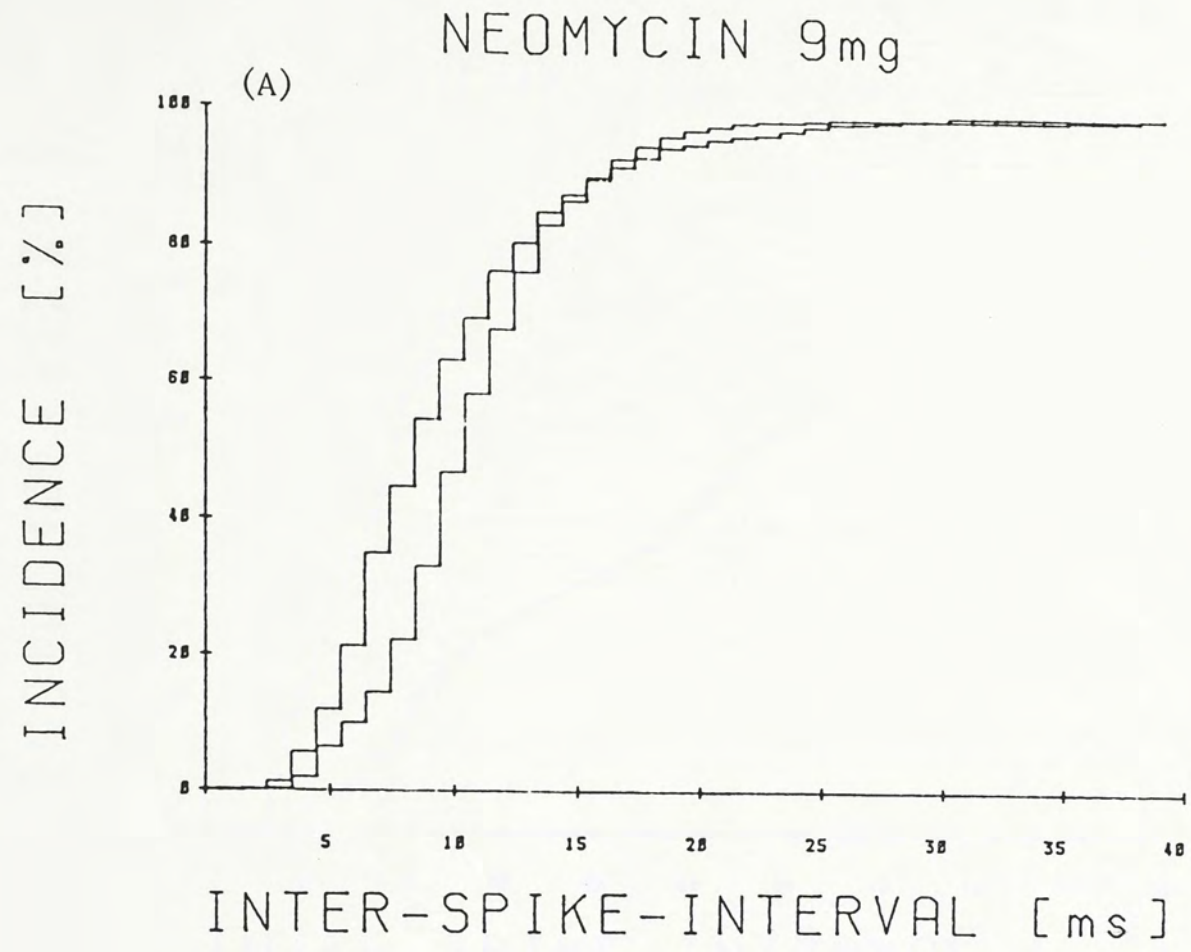
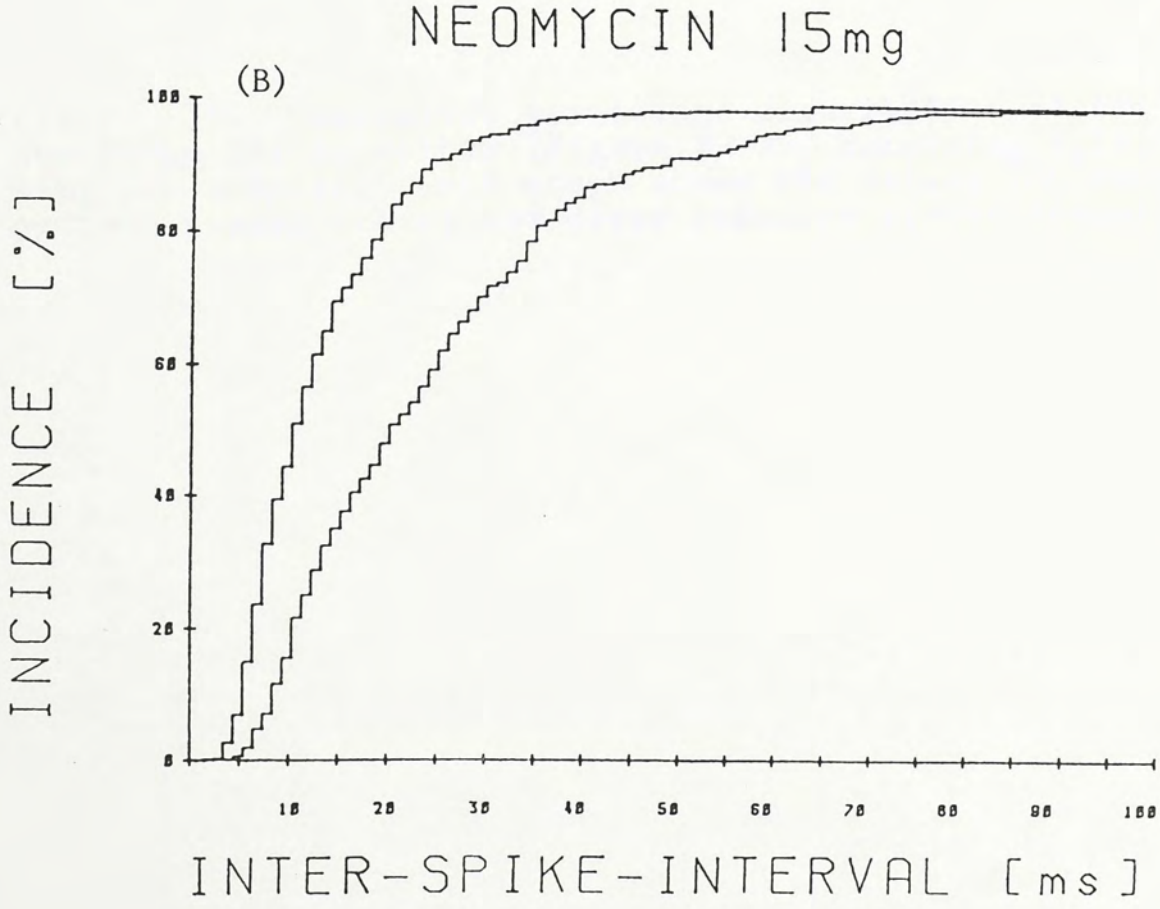


Figure 35. The percentage distribution of ISI of a typical guinea pig SAI receptor during dynamic response (1200-2200 ms). Data were obtained from 10 steady responses before (A) and 10 steady responses after (B) infusion of 45 mg of neomycin.


NEOMYCIN 9mg
(A)
INCIDENCE [%]
100
80
60
40
20
0
5
10
15
20
25
30
35
40
INTER-SPIKE-INTERVAL [ms]


NEOMYCIN 15mg
(B)
INCIDENCE [%]
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
100
INTER-SPIKE-INTERVAL [ms]

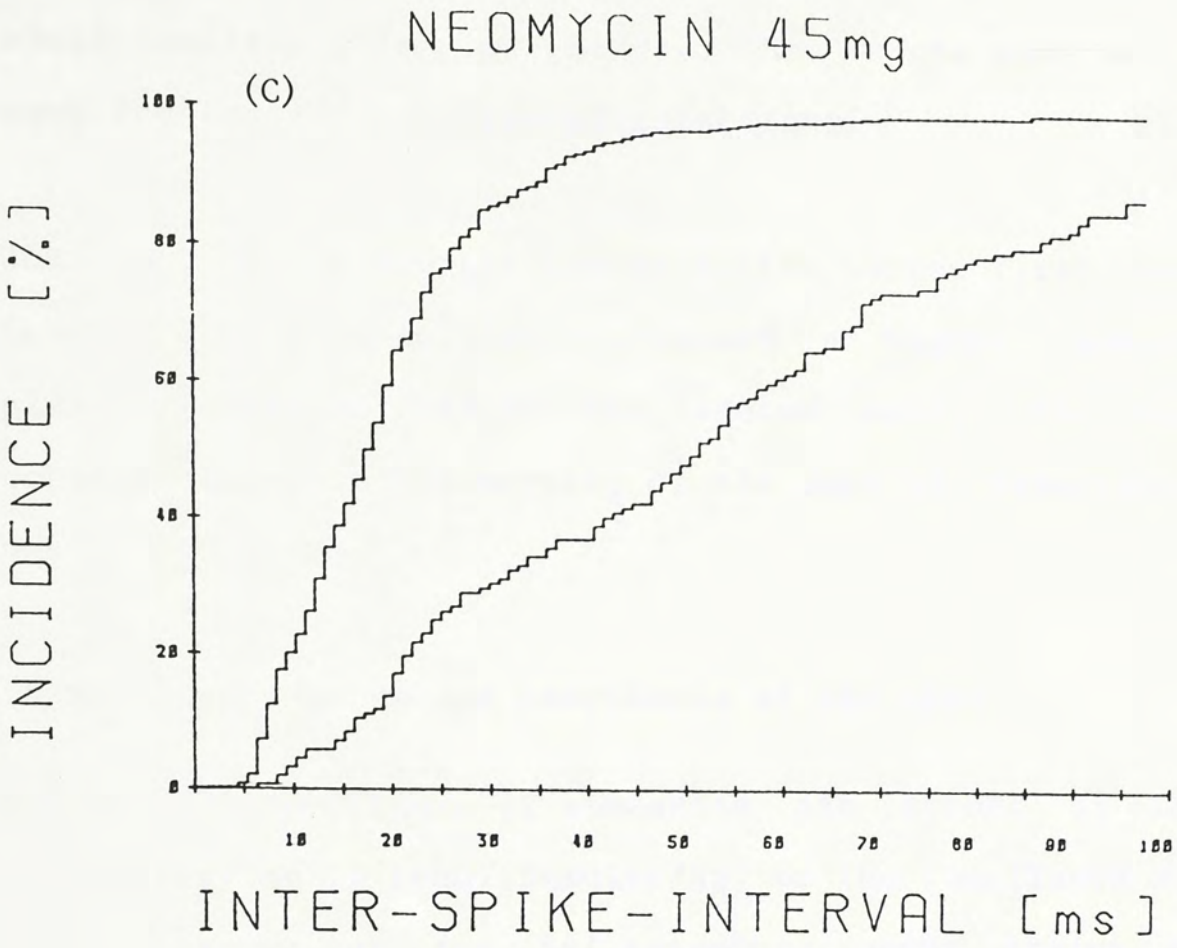


Figure 36. Cumulative percentage distribution of ISI of the three SAI receptors (Figure 33-35) receiving 9, 15, and 45mg of neomycin. Each graph shows the values for control periods (upper trace) and after infusion (lower trace).

about 5mmoles/kg body weight/day. This is the same amount used for the intraperitoneal injections.

The rats had a low Li+ concentration in the first week (about 0.4 mM) and later attained a higher constant plasma level of Li+ in the second week. The mean concentration on the morning of the days of experiments was 0.90±0.2mM.

(b)Nervous response and compliance of the skin

Figure 37 to Figure 42 summarize the effect of Li+ administration by food (5mmoles/kg) on the compliance and responsiveness of the SAI receptors under trains of stimuli of 15 and 20mN of constant force. Figure 37 shows that in control rats, the first stimulus evoked a mean number of impulse of 209±10 and the last stimulus 23±1. Li+ did not alter the responsiveness of the SAI receptors to 15mN constant force for any of the 40 stimuli given, from 222±7 in the first stimulus to 20±2 impulses. Concerning the dynamic and static responses shown in Figure 39A and 39B respectively, Li+ seemed to inhibit the dynamic response but not the static responses. Figure 38 shows that the compliance of the skin in terms of stroke amplitudes was not affected. For 20mN stimuli, the total response (Figure 40), dynamic response (Figure 42A), static response (Figure 42B) and the compliance of the skin did not show great differences

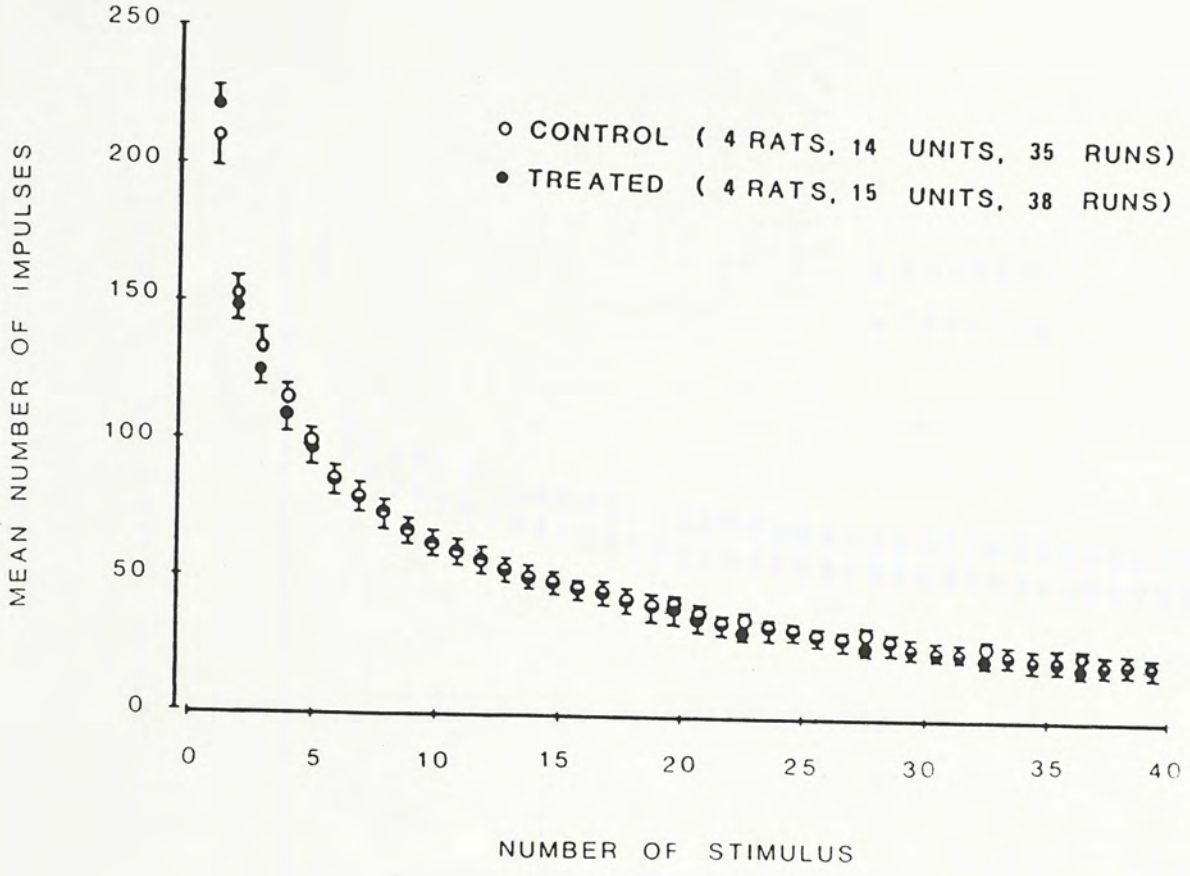


Figure 37. Responsiveness of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (15±0.5mN) plotted against ordinal number of stimulus for control and Li-fed rats.

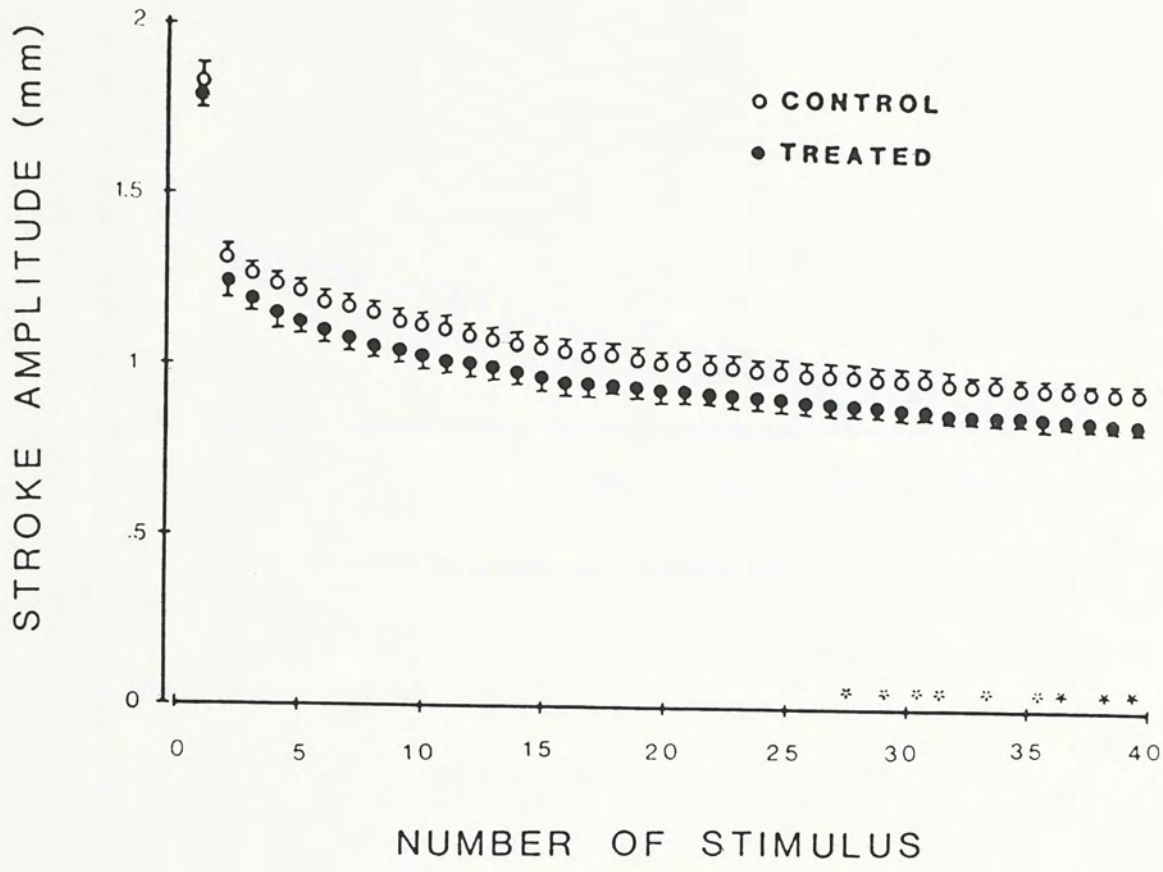


Figure 38. Values of stroke amplitudes (maximal indentation - residual indentation) of control and Li-fed rats plotted against ordinal number of stimulus for trains of 40 constant force stimuli of 15±0.5mN

Figure 39. Dynamic response (0-250ms)(A) and static response (1200-2200ms) (B) of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (15±0.5mN) plotted against ordinal number of stimulus for control and Li-fed rats. Significant differences of corresponding values between groups are indicated by asterisks.

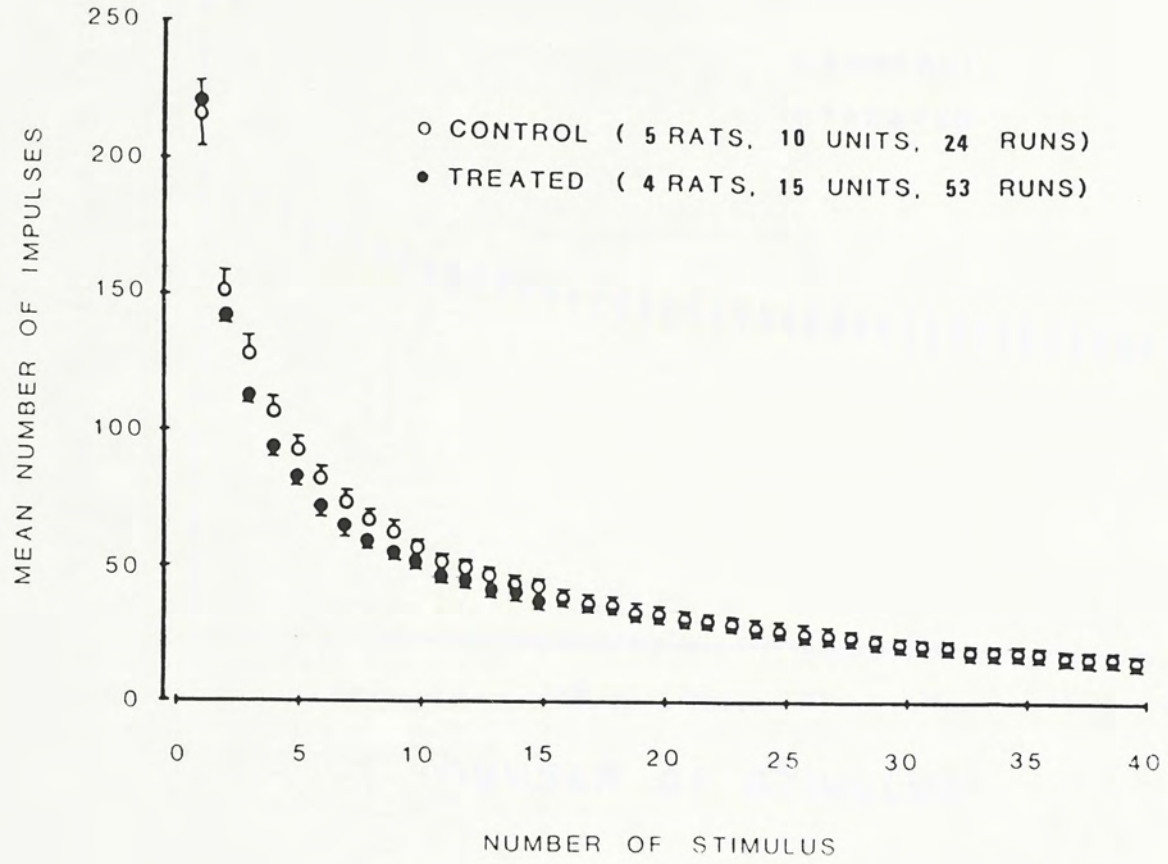


Figure 40. Responsiveness of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (20±0,5mN) plotted against ordinal number of stimulus for control and Li-fed rats.

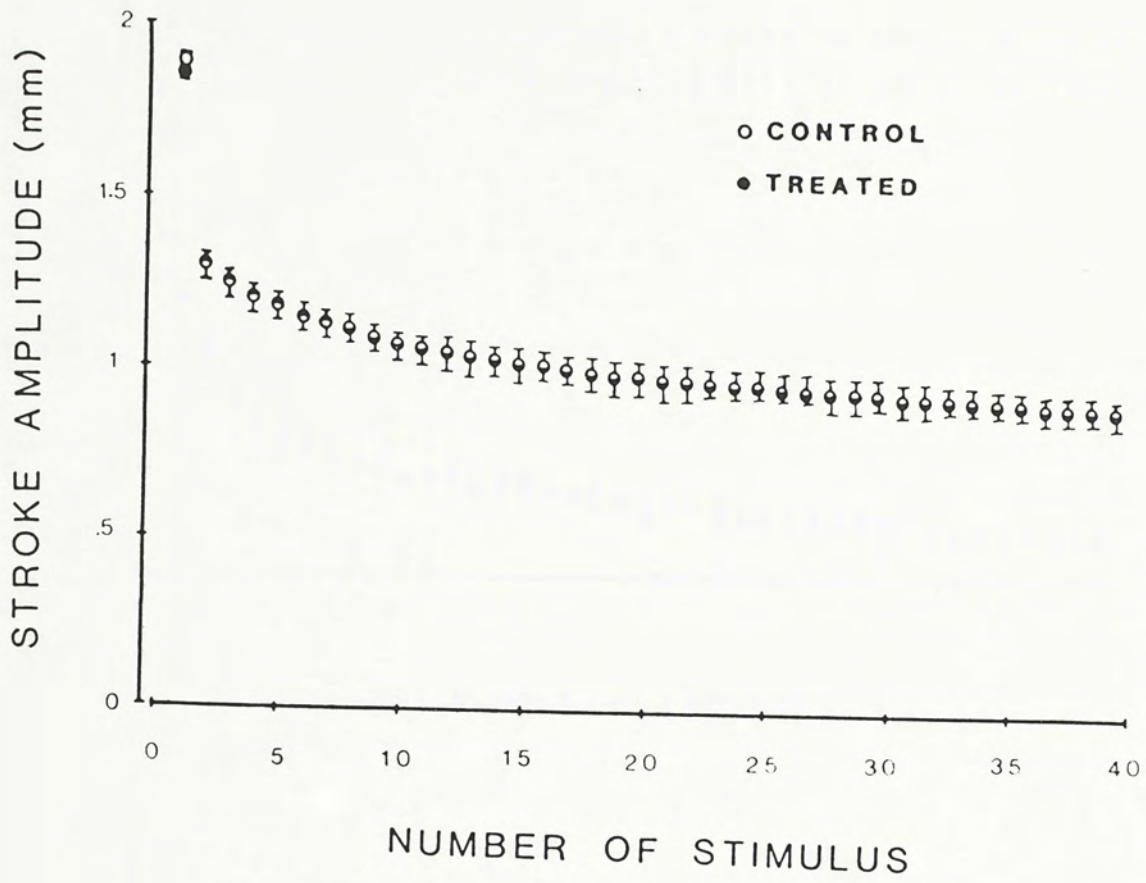


Figure 41. Values of stroke amplitudes (maximal indentation - residual indentation) of control and Li-fed rats plotted against ordinal number of stimulus for trains of 40 constant force stimuli of 20±0.5mN.

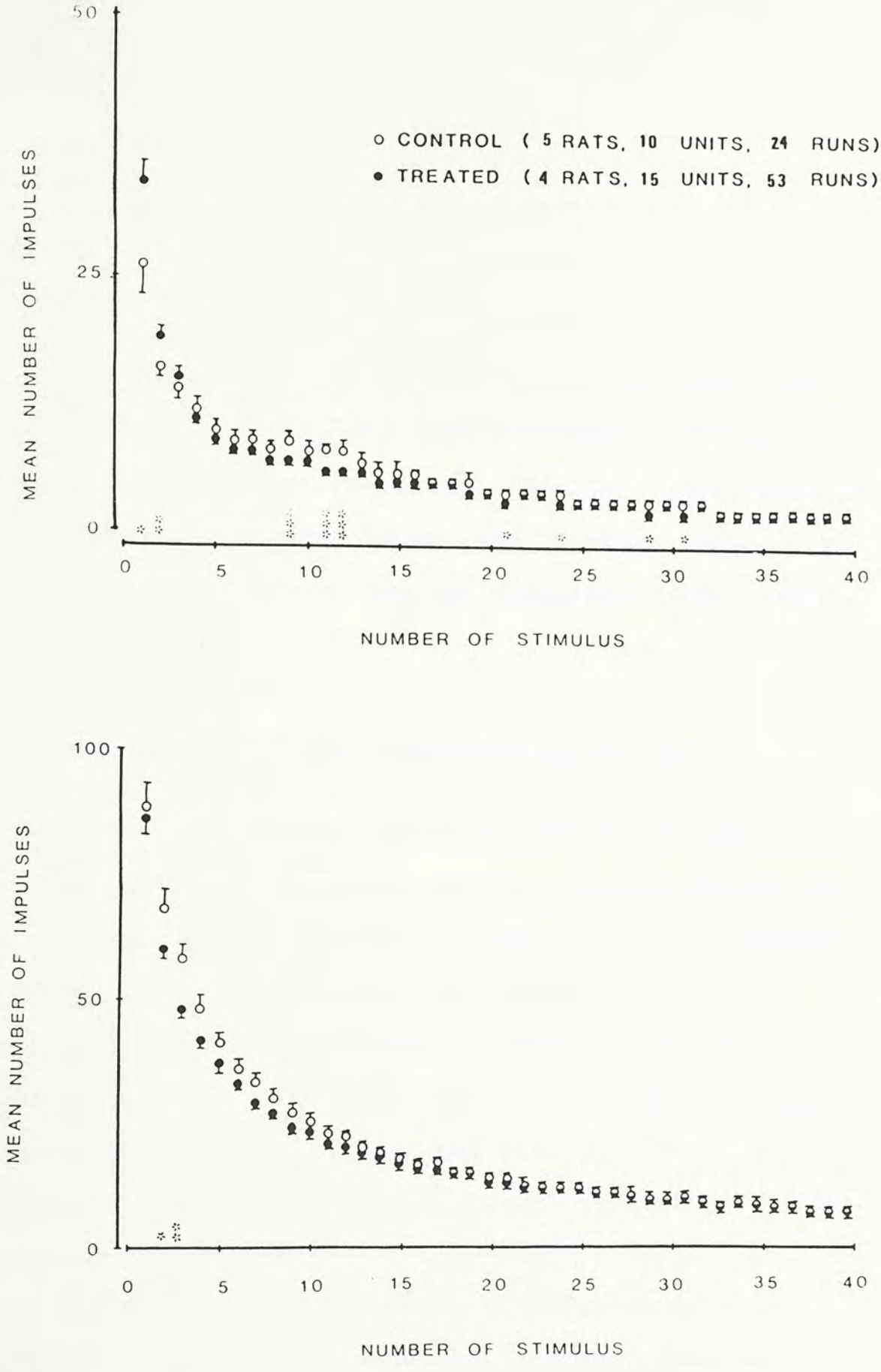


Figure 42. Dynamic response (0-250ms) (A) and static response (1200-2200) (B) of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (20±0.5mN) plotted against ordinal number of stimulus for control and Li-fed rats. Significant differences of corresponding values between groups are indicated by asterisks.

(Figure 41).

3.3.2 By I.P. injection

(a)General condition of the rats

The administration of Li+ by I.P. injection also did not affect the body weight and food consumption of the rats compared with the control group. Similar increases in water consumption were found. The average plasma Li+ levels obtained were 1.9±0.1mM measured at the end of experiment.

(b)Nervous responses and compliance of the skin

In this study the nervous responses of the receptors were affected by Li+. Responses in control rats ranged from 218±10 impulses for the first stimulus to 23±1 impulses for the last one with the 15mN (Figure 43) force of stimulation. Corresponding control values for stimulation with 20mN were 207±15 and 19±2 impulses (Figure 46). In rats treated with Li+ there was no difference for responses to the first stimulus (15mN). In response to 20mN stimuli the first response in the train of 40 was 19.3% higher in Li+ treated rats than in controls. Subsequent responses became progressively smaller than their matching controls from the second response (14.9% to 47.8%) onwards in the 15mN sequence and starting with the third response (3.7% to 26.3%) for

the 20mN stimuli. The compliance of the skin was not affected as shown in Figure 44 and 47. Responses in the dynamic (Figure 45) and static (Figure 48) phases followed closely the results of the total responses. In rats given 20mN stimuli, the dynamic phase was less affected than the static phase (Figure 48).

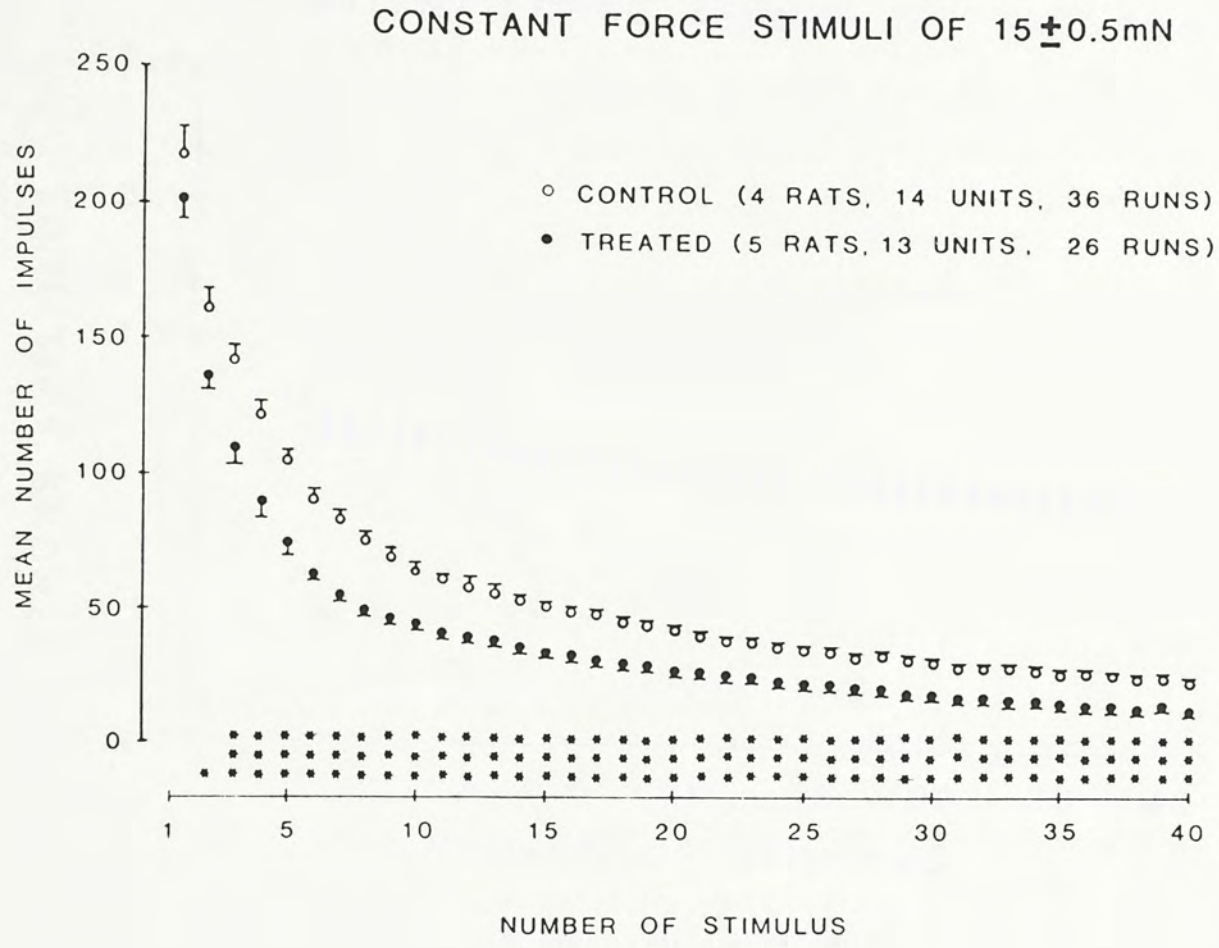


Figure 43. Responsiveness of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (15±0.5mN) plotted against ordinal number of stimulus for control and Li-treated (i.p.) rats. Significant differences of corresponding values between groups are indicated by asterisks.

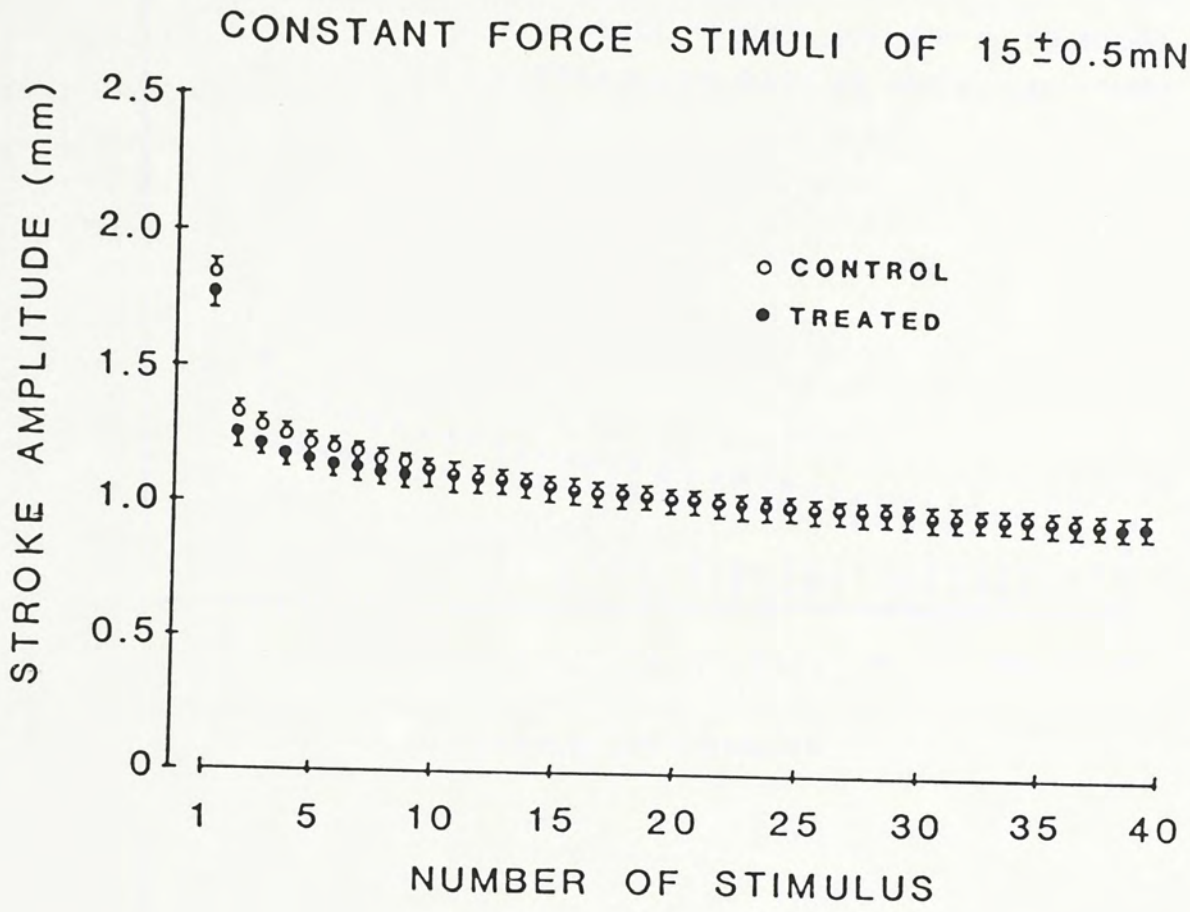
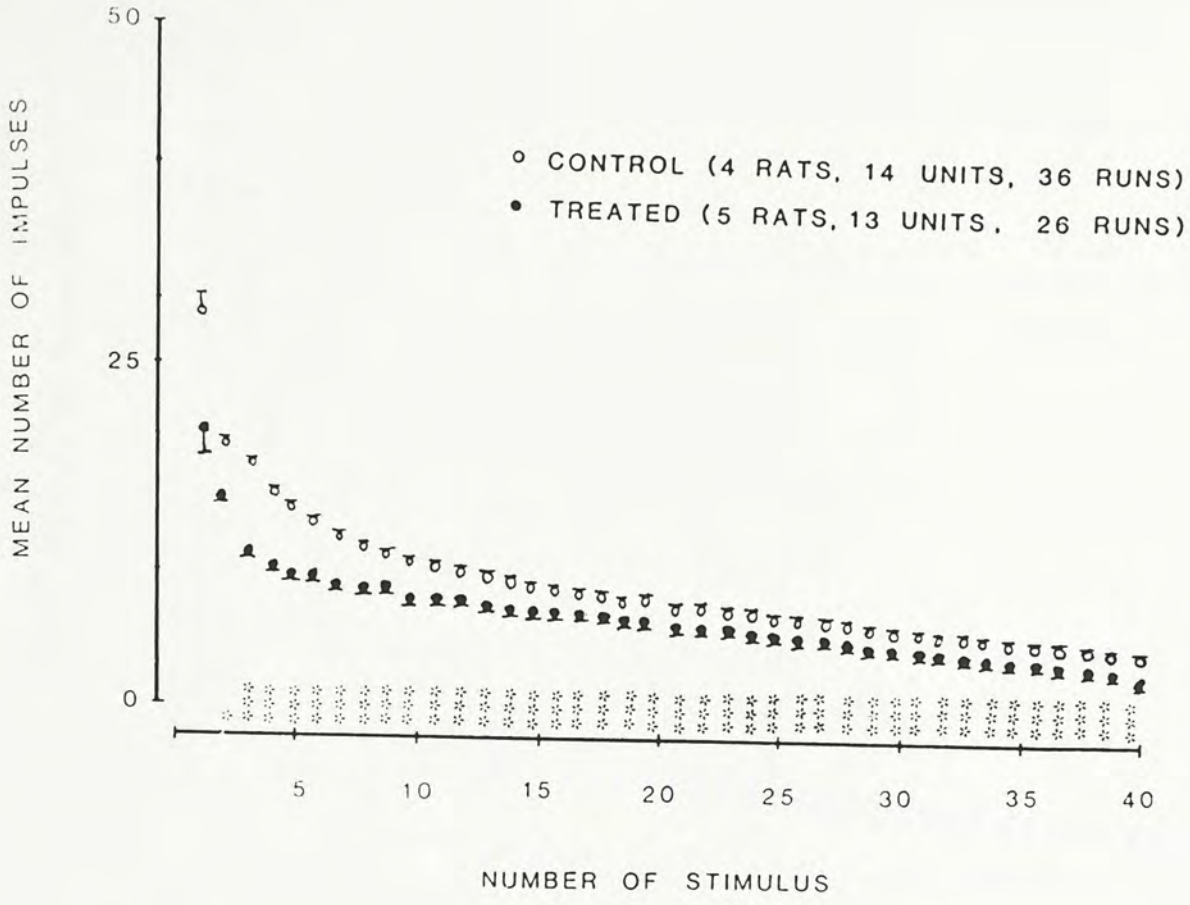


Figure 44. Values of stroke amplitudes (maximal indentation - residual indentation) of control and Li-treated (i.p.) rats plotted against ordinal number of stimulus for trains of 40 constant force stimuli of 15±0.5mN.



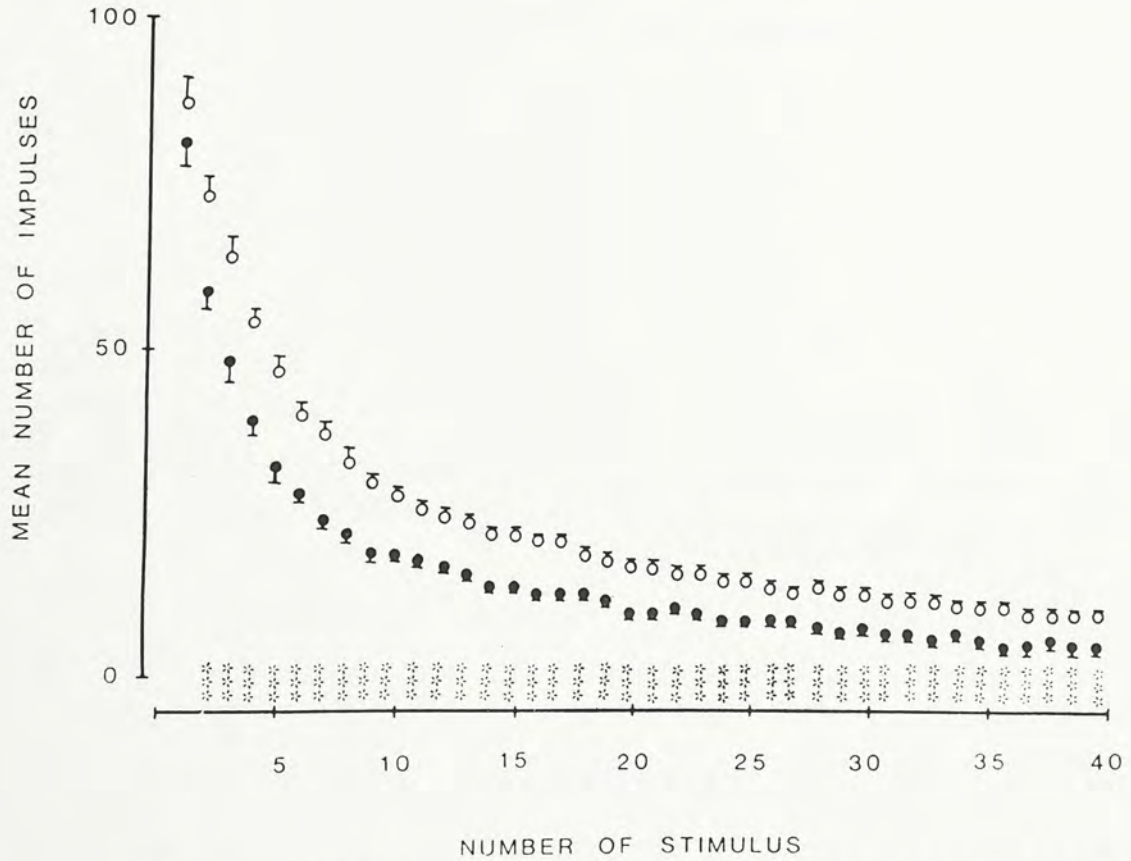


Figure 45. Dynamic response (0-250ms) (A) and static response (1200-2200ms) (B) of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (15+0.5mN) plotted against ordinal number of stimulus for control and Li-treated (i.p.) rats. Significant differences of corresponding values between groups are indicated by asterisks.

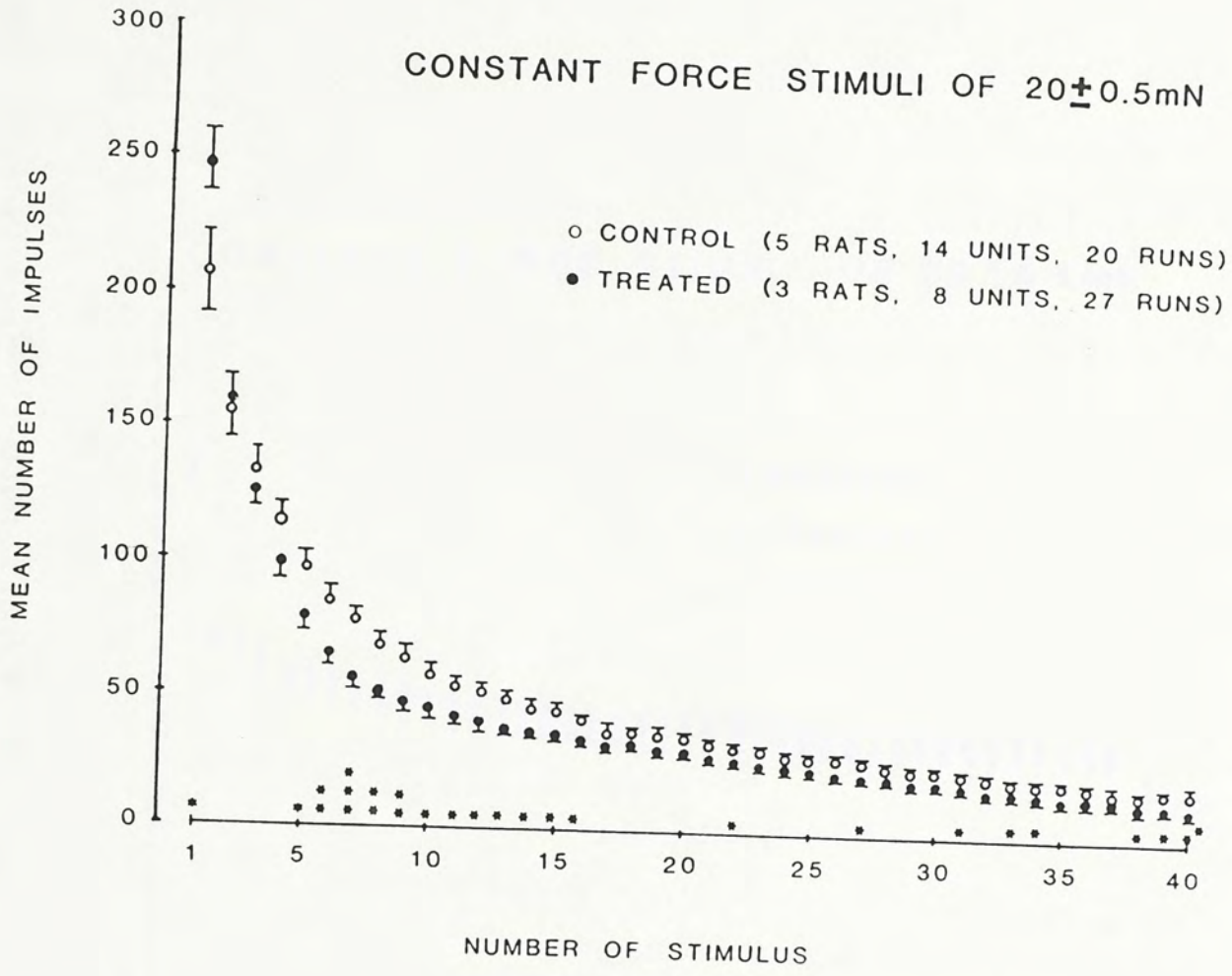


Figure 46. Responsiveness of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (20±0.5mN) plotted against ordinal number of stimulus for control and Li-treated (i.p.) rats. Significant differences of corresponding values between groups are indicated by asterisks.

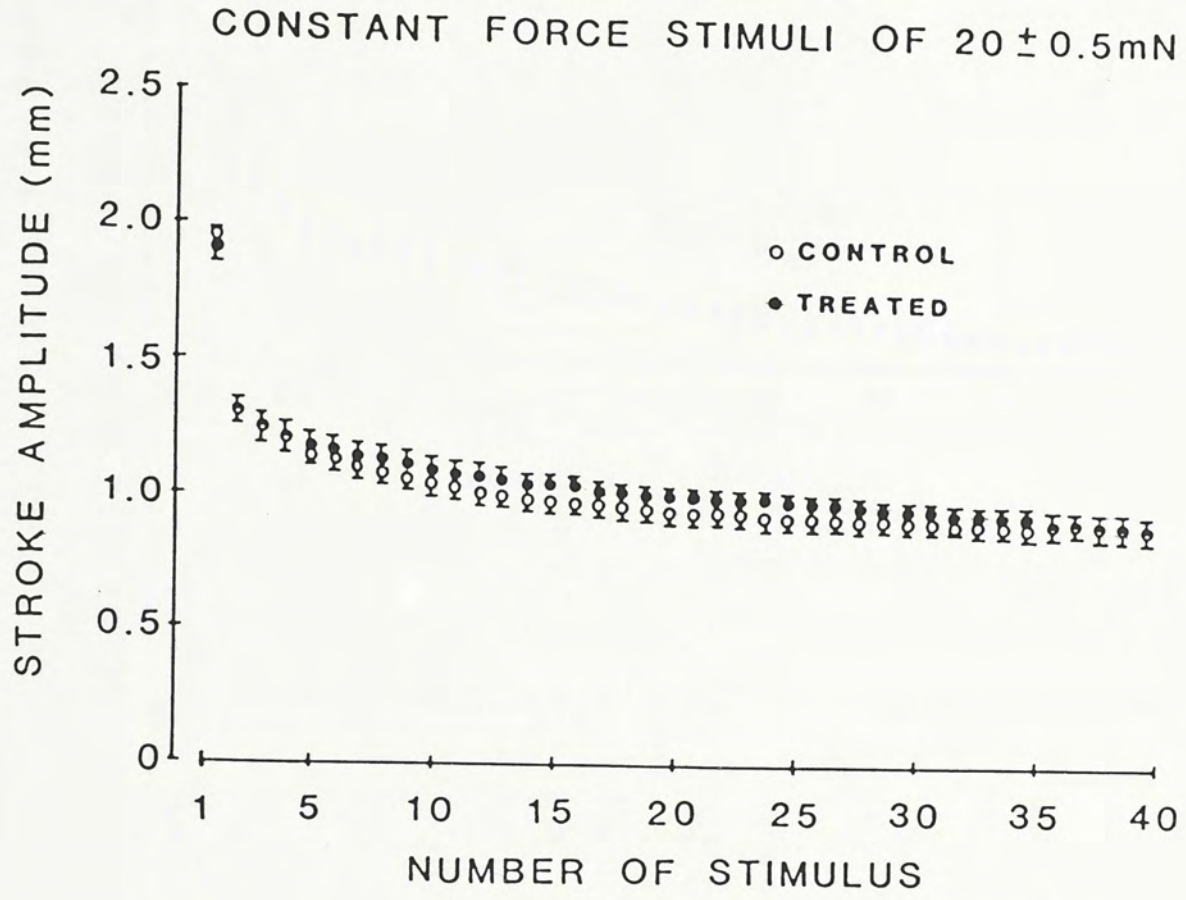
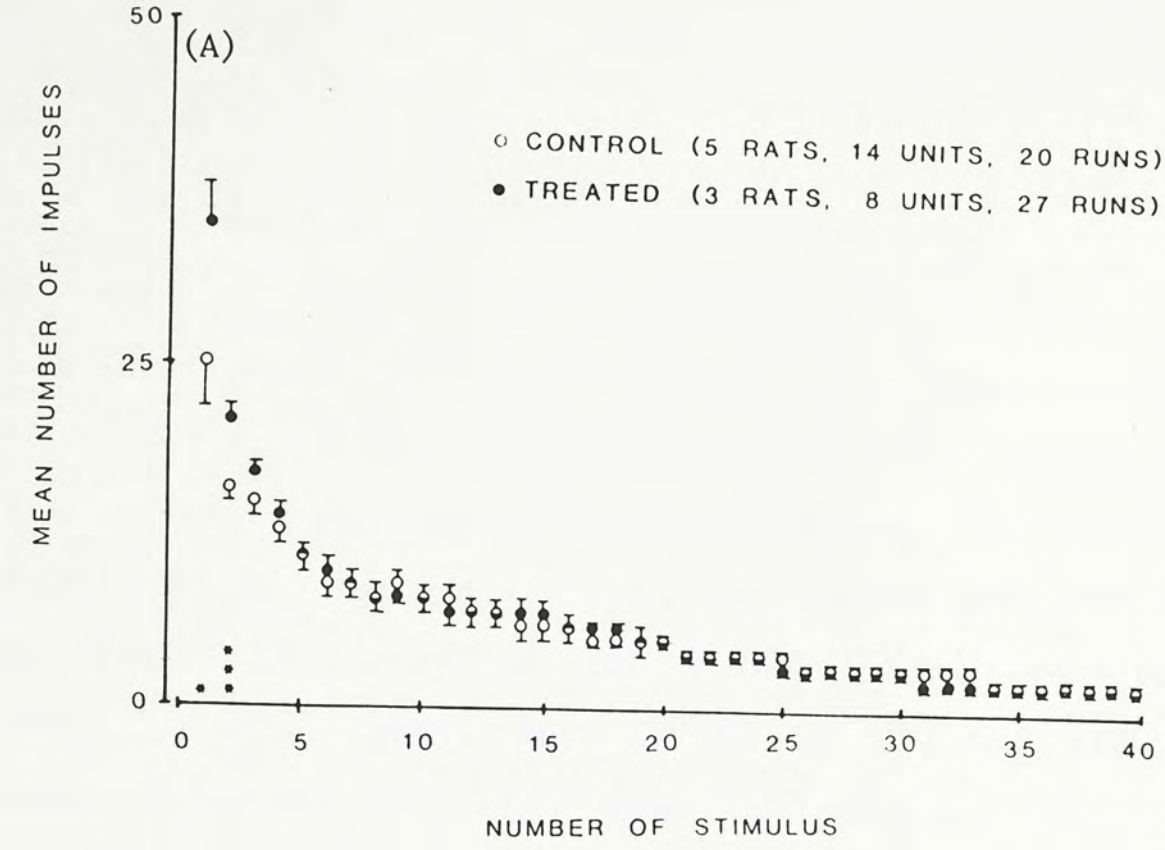


Figure 47. Values of stroke amplitudes (maximal indentation - residual indentation) of control and Li-treated (i.p.) rats plotted against ordinal number of stimulus for trains of 40 constant force stimuli of 20±0.5mN.



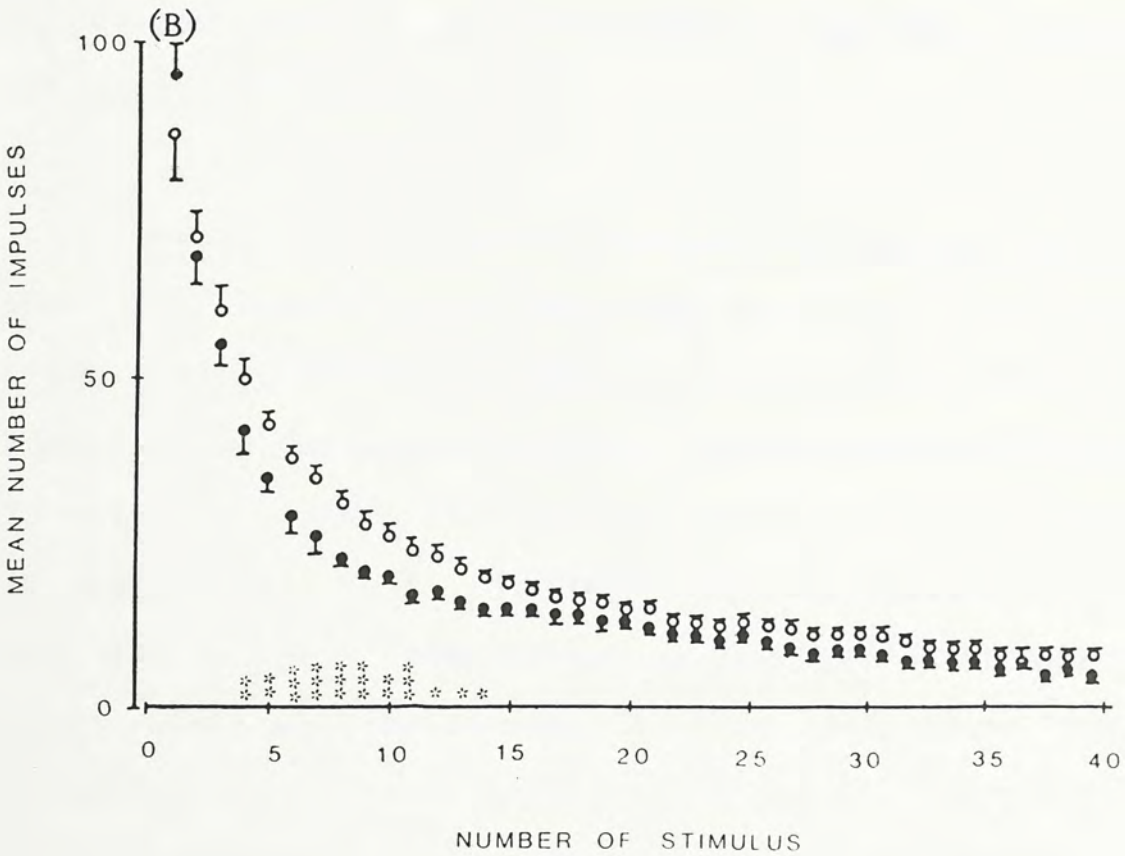


Figure 48. Dynamic response (0-250ms) (A) and static response (1200-2200ms) (B) of SAI receptors (in mean number of nerve impulses per stimulus) to trains of 40 constant stimuli (20±0.5mN) plotted against ordinal number of stimulus for control and Li-treated (i.p.) rats. Significant differences of corresponding values between groups are indicated by asterisks.

SECTION 4. DISCUSSION AND CONCLUSION

4.1 Functional Properties of Guinea Pig SAI Receptors

The present experiments show that Merkel cell-neurite complexes in the hairy skin of guinea pig exhibit functional properties similar to those of SAI receptors found in other species such as the rat, cat and rabbit. However, there are also some differences. In the following discussion, emphasis will be put on the comparison with other well-studied species, which is summarized in Table 1.

The receptors in different species are similar in that they all exhibit slowly adapting behaviour. In the cat (Iggo & Muir, 1969) and rabbit (Aitken & Lal, 1982), upon a maximum stimulation, the unit can discharge for up to 30 minutes. In guinea pig SAI receptors, the maximum discharge duration has not been systematically tested but one SAI receptor under investigation did discharge for more than 6 minutes showing its long adaptation phase. The mechanical thresholds of guinea pig SAI receptors are characteristically low. When compared with those of cat and rabbit in the same region, the mean force threshold and displacement threshold are of the same order of magnitude. The rat shows a higher force and displacement

	Guinea Pig	Cat	Rabbit	Rat
Morphology	Domes not clear	Clear domes	Clear domes	Clear domes
Receptive Field Property	concentrated but not always punctate	punctate	concentrated but not always punctate	punctate
Displacement threshold	<25um	1-5um	4-30um Mean 13+4.2um	
Force Threshold	<1mN	<0.1mN		1.1+0.1mN
Resting Discharge	Rare, low frequency and irregular	rare	rare	no
Response to Stretch	usually respond to vigorous stretch	rare unless severe and prolonged		
Force-encoding Function	Linear			
Displacement-encoding Function	Power n=1.6	Power	Linear	
Off-response	no	no	1-2 spikes characteristic	no
Adaptation	2 time constants: 0.24-3.8s; 10.1-384.8s	2 time constants: 0.5-0.6s; 10-20s		
Coefficient of Variation	0.70-1.05	>0.5		
Minimum ISI	1ms	<1ms		
ISI distribution fitted by	Gamma of k=1 or 2	Truncated Poisson		
References		Iggo & Muir, 1969	Aitken & Lal, 1982	Leung, 1986

Table 1. Comparisons of properties of SAI receptors in hairy skin of 4 different species.

threshold probably due to the different skin area tested.

The receptors usually do not exhibit resting discharge, which is a characteristic distinguishing them from SAII receptors. In addition, all SAI receptors of the different species are not responsive to stretching. However, severe stretching of the skin area containing the SAI receptors can elicit nervous discharge. Since some of them show directional preferences, it is reasonable to assume that the surface morphology of the receptors may determine their response to stretching and their directional preferences. This may be especially true in the case of the guinea pig SAI receptors because unlike those of cat, rabbit and rat in which SAI receptors appear as distinct domes on the skin surface, the guinea pig hairy skin does not show such distinct domes.

The guinea pig SAI receptor responds typically to mechanical stimulation with both a dynamic and a static phase of mechanical stimulation. The receptors discharge in a burst of high frequency in response to the dynamic phase of a stimulus. By analysing the ISI distribution during the early phase of a stimulus, the minimum ISI obtained was 1 ms, but that was very rare, indicating that the receptors seldom discharge up to 1 kHz, even under maximal stimulation. This is different from that of cat SAI receptors which have been reported to have a

discharge that can be higher than 1 kHz (Iggo and Muir, 1969). A minimum ISI of 2ms, on the other hand, is more common in the dynamic response. It may be due to the lower ramp used.

The adaptation behaviour of SAI receptors of different species also show slightly different values. The cat, for example, was reported to have 2 exponentially decreasing phases consisting of 2 relatively short time constants. (Iggo & Muir, 1969). However, in guinea pig, the usual pattern, as reported in Results section, is that during the first 5 seconds, a single negative exponential phase occurs which is then followed by a phase with a longer time constant. A smaller time constant within the first second which is comparable to that found in the cat cannot be excluded but the experimental data can well be fitted with only one time constant for the first 5 s. The discharge of the receptor in response to the sustained stimuli approximates a steady state. It is this steady state the statistical modelling of the ISI distributions was carried out upon because most of the statistical analysis of probability distribution assumes a steady state discharge. But it has to be borne in mind that this analysis is based on a reasonable approximation only.

Previous studies on the amplitude coding behaviour of SAI receptors have shown that the response is commonly

related to the velocity and amplitude of indentation by a power function (Iggo and Muir, 1969; Aitken and Lal, 1982). There has been relatively little information on the response in relation to the force applied. A constant displacement stimulation usually results in an overshoot of force followed by a slow decline of force (Pubols, 1982; Hamann and Lee, 1982). The pattern of force is less well characterized and quantitated. A constant force stimulus, however, yields both a steady increase in the values of force and amplitude during the dynamic phase. While the force remains steady during the static phase, the amplitude increases slightly (Pubols & Maliniak, 1984; Baumann, Hamann & Leung, 1986b). The present study indicates that the response is linearly related to the force applied but related to the displacement amplitude by a power function. The findings agree with previous studies on other species.

Although the mean frequency or total counts of a response to a particular stimulus can yield useful information, the variability of the ISI values has been concealed. Therefore, some measures have been taken in the past to characterize variability of spike generation of receptors. These include the coefficient of variation which gives a measure of the variability of the ISI relative to the mean.

In the fully adapted state, the cat SAI receptors have

coefficients of variation greater than 0.5, in contrast to SAII receptors which have very low CV of less than 0.3 (Chambers et al., 1972). In the footpad of cat (Ferrington, 1985) the change in CV at different time periods have been recorded. During the dynamic state, the CV fluctuates and usually has higher values than the relatively stable CV at later stages. The later CV tested in cat footpad have a CV greater than 0.5. In the present study, the relatively high CV of the SAI receptor during adapted stage (some units have a CV approaching 1) indicates that the ISI values are more variable than those found in cat or rabbit.

A truncated Poisson distribution has an appearance similar to Gamma distribution with a low shape parameter. Since nearly all the ISI distributions obtained in guinea pig units can be described by a Gamma family with either 1 (Poisson) or 2 as the shape parameter, Gamma distribution may well provide a generalized statistical model for the distribution of the ISI of SAI receptors. The Gamma distribution has also been used to characterize a number of point processes such as neurotransmitter release (Boyd and Martin, 1956) and distribution of interval time between opening of ionic channels (Colquhoum and Sakmann, 1985).

The interpretation of a truncated Poisson is different

from that of a Gamma distribution. In the first case, a reasonable explanation of the ISI distribution pattern has been discussed by Iggo (1976) by assuming that the absence of very short intervals is due to the resetting phenomenon of the afferent terminals in a group of Merkel cells inside a dome.

A Gamma distribution with a shape parameter greater than 1, on the other hand, assumes that more than one factor contributes to the generation of a spike each of which affects it randomly and each having an exponential distribution. Therefore, any physical model based on Gamma distribution may represent an alternative to the truncated Poisson model. In fact, in the frog Type I units, Gamma distribution of the values of larger parameters can be made to fit the ISI distribution to a reasonably good extent. In no ways can these ISI distributions be described by a simple Poisson process. (Ogawa, 1982)

4-2 POSSIBLE INVOLVEMENT OF PHOSPHOINOSITIDES IN MAMMALIAN SAI RECEPTORS

The finding of Schacht (1974, 1976) that neomycin can bind tightly and selectively to polyphosphoinositides forms the basis of the use of this antibiotic as a tool to study the possible involvement of PIP2 hydrolysis in

biological processes. For example, neomycin suppresses Ca2+-transients evoked by electrical excitation of skeletal muscle (Vergara et al., 1985). This observation supports the proposal of a second messenger role of IP3 in skeletal muscle and neomycin acts to inhibit the generation of IP3 by inhibiting phospholipase C-mediated PIP2 breakdown.

The idea of testing the possible effect of aminoglycosides on SAI receptors stems from the observation of the similarities between Merkel cell-neurite complexes and the hair cells of the acousticolateralis system, especially those in the inner ear of mammals. Both types are mechanoreceptors and have highly developed sensory cells and specially adapted terminals to the afferent nerve fibres. By proposing a mode of action of Merkel cell receptors similar to that of hair cell receptors, the present experiments tried to find out whether neomycin acts on SAI receptors in a comparable fashion.

Experiments on the action of neomycin have been done on rats after chronic neomycin administration (Baumann et al., 1984) and in cats using similar acute infusion of neomycin like in the guinea pigs of this study (Baumann et al., 1986a). Since guinea pig is the most extensively studied animal model of aminoglycoside ototoxicity, the SAI receptors of the guinea pig can be considered the

most appropriate model to test whether there are similar effects of aminoglycosides on hair cells and Merkel cell receptors.

The present experiment confirms the previous findings on rat and cat (Baumann et al., 1984; Baumann et al., 1986a), that is, neomycin strongly suppresses the responsiveness of the SAI receptors. If a chemical substance is known to affect the responsiveness of a cutaneous mechanoreceptor, it may exert its effect in several ways. First, it may affect the compliance of the skin and therefore the force transmission properties of the skin. Second it may affect the nervous function of the afferent nerve fibre. Third, it may affect the functioning of the sense organ itself.

Concerning the first point, chronic administration of neomycin to the rat caused a hardening effect of the skin (Baumann et al., 1984) and may therefore affect the interpretation of results. The acute close arterial infusion method employed in a study on cats (Baumann et al., 1986a) and in the present study enables us to observe the acute effect of neomycin without changes in compliance of the skin. The mechanical properties of the skin are not changed, as indicated by the result.

For the second point, conduction block is not a likely explanation of the the reduced response. Neomycin topically applied to a length of 1 cm of the desheathed rabbit sural nerve (350 mg/l) (Chan et al., 1985) and to isolated frog sciatic nerve (3000 mg/l) (unpublished observation) does not change the shape or conduction velocity of the A-action potential elicited electrically. Concerning the third point, neomycin has been found to affect the ultrastructure and affect the respiratory function of the Merkel cells (Cheng-Chew et al., 1985). These observations support the notion that neomycin affects the Merkel cell-neurite complexes directly.

However, there are two possible ways by which neomycin affects the receptors: 1)It may act as Ca2+ -entry antagonists, in a manner similar to that during the suppression of mechano-electric transduction observed in hair cell receptors (Ohmori, 1985) or by impairing the putative synaptic transmission in Merkel cell-neurite complexes, as observed in several neuromuscular junction models (Prado et al., 1978). In Ohmori's study, the inhibitory effect of neomycin and streptomycin is voltage dependent: the reduction of the transduction current in isolated vestibular hair cells of the chick becomes larger with hyperpolarization of the membrane. This suggests that the positively charged antibiotic molecules may plug the mechanically gated channels. 2)it may also affect the polyphosphoinositide turnover by

interaction with the membrane PIP2 which is to be hydrolysed during signal transduction. The existence of phosphoinositides in hair cells and the inhibitory effect by neomycin has been found and discussed in Section 1.2.3. If such a system exists in the Merkel cell-neurite complex similar to that in the hair cells then neomycin may also inhibit the responsiveness by preventing the PIP2 hydrolysis. This effect is expected to be acute and should depend on the concentration of neomycin used since the PIP2 is located in the plasma membrane of the cells.

The neomycin effect on SAI receptors is dose-dependent, as has also been found in the cat (Baumann et al., 1987). In cats, responsiveness of SAI units recovers very slowly after it has been suppressed by neomycin. In the case of guinea pig usually there is no recovery of the responsiveness. This shows that guinea pig SAI may be more susceptible to the effect of neomycin than cat SAI receptors. The effect observed is not a general toxic effect of neomycin in the circulation because the method of infusion allows neomycin to enter the limb circulation first before it returns to general circulation. The drop in responsiveness occurs usually within 1 or 2 minutes after neomycin enters the limb circulation.

Prentki et al. (1986) have questioned the specificity of neomycin in the study of the inositol-phospholipid signalling system. They found that neomycin can bind directly to IP3 and ATP in addition to PIP2. In other words, the suppressive effects of neomycin may be explained by this phenomenon. However the finding does not invalidate the usefulness of neomycin in these studies because its interaction on IP3 means that it has more than one site of action on the PIP2-IP3 system. If the Merkel cell uses IP3 as second messenger, the effect of neomycin on IP3 itself would give rise to the expectation that its effect should be even more pronounced. It remains to be determined whether the effect of binding ATP alone can cause the inhibition of cellular responses to the same extent.

In summary, neomycin suppresses responsiveness of SAI receptors by a direct action on the sense organ itself. At least two possible mechanisms can explain the effect: 1)a cationic effect and 2)an effect on the signal transducing capacity of the phosphoinositide turnover mechanisms that may exist in the SAI receptors. The two mechanisms, however, do not exclude each other.

It must be noted that the present evidence is indirect. The proof of the existence of phosphoinositide system requires ultimately an in vitro study that allows full control of the environment of the receptors.

The study of Lithium effects enables us to look at the problem from another angle. Preliminary tests show that acute infusion, either in the rat or in the cat, does not alter the sensitivity of the SAI receptors, for up to three hours after infusion, either by a bolus injection in the rat or by close arterial infusion in the cat. The present experiments were therefore carried out to study the effect of the chronic administration of Li+. The two modes of Li+ administration were employed to study whether the route of administration (which in turn affects the profiles of the plasma levels) may produce a difference in the effect of Li+.

A preliminary test showed that Li+ increased the first response of 30 consecutive constant force stimuli but decreased later responses. However, due to the toxicity of Li+, which had been maintained at a high concentration, the rats were dehydrated which caused hardening of the skin and the rats developed toxic symptoms. The present experiments therefore aimed at giving as high a dose of Li+ in vivo as possible without inducing toxic symptoms. The results showed that the present dosage regimens, either by feeding or by intraperitoneal injection, did not cause the development of any observable toxic symptoms. Li-fed and Li-injected rats developed polydipsia and polyuria as a common side effect of Li+ (Baldessarini, 1985). Since the body

weight of the treated group did not differ significantly from control, it was assumed that the rats had adapted to the side effects and toxic symptoms did not develop.

Although the two dosage regimens did not show a difference in their effects on the general state of the rat, they did result in different behaviour of the receptors in response to constant force stimuli of 15 and 20 mN. Oral administration of Li+ was not effective in causing significant changes in the nervous response, either during the dynamic phase or the static phase. The compliance of the skin, as seen in Results section, was also not affected.

Li+ administration by I.P. injection, however, caused varying levels of Li+ in the body. It has been shown in the rat by Plenge et al. (1981) that the sort of Li+ administration used in the present study causes an abrupt increase in serum Li+ level to a very high level which then declines exponentially to a low concentration before the next injection. Although the plasma Li+ concentration during stimulation has not been measured, the concentration of Li+ immediately after each experiment had a mean value of 1.9 ± 0.1mM. This concentration was about twice that observed in experiments on rats using oral administration. The Li+ concentration profile of the rats after receiving an

injection of Li+ was not studied but by reference to another study using similar dosage of Li+ level (Plenge et al., 1981), the range of Li+ level should fall in the range from 1.9 to 2.3mM during the stimulation period of the SAI receptor.

From the two studies it seems that the general inhibitory effect of Li+ depends on the pattern of Li+ concentration. Since the dose was the same in the two treatments, Li+ may exert its effect by reaching a high concentration in the plasma. Various studies in the past have been done to compare the effectiveness of daily divided dose of Li+ with a single daily dose. The former yields a rather constant concentration of Li+ while the latter produces a fluctuating concentration of Li+. While the average steady-state Li+ serum concentration is unchanged from divided daily dose to single daily dose (Perry et al., 1981), the former pattern seems to be more harmful to the kidney (Plenge et al., 1981; Plenge et al., 1982). With regard to therapeutic efficiency of Li-treatment, there seems to be no difference between the two routes of application. It was thought that that peak values may be harmful but this idea is not supported by these and the present studies. On the contrary, they emphasize the importance of reaching low serum concentrations regularly. However, concerning other effects, I.P. injection produces more pronounced effect

on increase in water intake and polyuria than Lithium application through gastric tube (Olesen et al., 1976). Therefore there is no general answer to the question whether Li+ exerts the strongest effect in a highly variable cyclic pattern or constant level pattern . It may vary from one organ to another and from one Li+ effect to another.

The effect of Li+ on SAI receptors is consistent with the hypothesis that a degrading IP3 enzyme mechanism exists in the Merkel cell-neurite complex. Li+ inhibits the enzyme myo-inositol-1-phosphatase so that the cycle is stopped at the final step of inositol regeneration.

One could speculate in more detail how Li+ affects the excitability of SAI receptors. This can be based on the following discussion as to how the sensitivity of receptor cells with a PIP2-IP3 transducing mechanism can be affected by biochemical lesions.

If one of the primary events of the receptor mechanism is PIP2 hydrolysis, the sensitivity of the receptor mechanism will depend on the availability of PIP2, which could be altered by a number of different factors. One way of altering the level of PIP2 is to adjust the equilibrium that exists among the phosphoinositides, the level of which is set by the balance existing between the kinases which phosphorylate PI to PIP2 and the

phosphomonoesterases which convert these two PPI back to PI. Subtle alterations in the activities of these opposing reactions will shift the position of the equilibrium, resulting in more or less PIP2. Any change that shifts the position of the equilibrium to the right, for example, may lead to supersensitivity. Another way of altering the concentration of PIP2 is by changing the overall concentration of the PPI within the membrane. The first indication that the size of the agonist sensitive pool might be important in determining receptor sensitivity emerged from studies on the blowfly salivary gland (Berridge & Fain, 1979) in which the size of the pool declines only after a high dose of 5-HT. A critical factor for keeping this agonist sensitive pool fully charged is the rate of the synthesis of PI.

Cells require relatively high concentrations of inositol for PI synthesis. In nerve endoneurium, a small reduction in the concentration of inositol causes a large change in oxygen consumption, which returns to normal when the concentration of inositol is restored (Simmons et al., 1982). Further evidence for this idea comes from studies on regenerating liver (Huerta-Bahena & Garcia-Sainz, 1983). A high concentration of extracellular inositol seems to be essential for cells to maintain the sensitivity of α1-adrenergic receptors for signal transduction.

As discussed in 1.2.2, Li+ inhibits the production of inositol originating from the inositol cycle and also depresses uptake from plasma. This severe disruption of inositol metabolism in cells by lithium will lead to a decline in the concentration of the phosphoinositides. When Li+ is first administered, it will have little effect since the cells start off with a normal pool of phosphoinositides which can be used for signal transduction. However, as this pool is used up and is not replaced because lithium blocks the production of inositol, it will begin to decline, resulting in a decrease in the effectiveness of the receptors. In the present experiments acute infusion of Li+ did not cause any change in responsiveness of the receptors. This observation that inhibitory action of Li+ takes time to develop is entirely consistent with clinical observations. The result is therefore a partial inhibition of the inositol cycle.

However, to interpret the Li+ effect on SAI receptors, we must take into account the possible effect of Li+ on cell membranes. Effects of Li+ on membrane properties involve its ability to mimic other biologically important cations such as Na+ and K+ (Ehrlich & Diamond, 1980). Li+ induces membrane depolarization, a decrease in action potential amplitude and an elevation of free extracellular K+ concentration. The basic mechanism

underlying these observations seems to be a shift of the K+ equilibrium potential as a result of an interaction of Li+ with the transport function of the Na/K pump. Li+ also affects synaptic transmission . For example, Li+ at 15mM increases the frequency of spontaneous activity in the isolated spinal cord of the frog (Grafe et al., 1982). In amphibian neuromuscular junctions, replacing Na+ in Ringer solution by Li+ first increases the MEPP frequency but at a later stage interferes with the transmission of action potential (Crawford, 1975). However, this Li+ effect is usually seen at a higher concentration and the effect cannot account, in the present situation, for the initial increase in response under a stronger stimulation (20mN). In the present study, this effect can only play a negligible role.

On the other hand, the phenomenon of an initial increased response to 20mN stimuli can be explained by the PIP2-IP3 signalling system. The chronically PIP2 depleted receptor has a general subsensitivity, as seen in the reduced response to 15mN stimuli. However, when a strong stimulus is applied, due to the presence of Li+ in the cell the depletion of IP3 is blocked and the concentration of intracellular IP3 increases and amplifies the response. This situation would be similar to the well known effect of Li+ in in vitro study. Li+ amplifies the agonist-stimulated accumulation of IP3 (5 to 20 fold) in blowfly salivary gland, rat brain slices,

and rat parotid gland (Berridge et al., 1982), and has been exploited in the pharmacological characterization of several receptors (e.g. Akhtar & Abdel-Latif, 1984; Albert et al., 1984) . It has been found that Li+ also inhibits the dephosphorylation of IP3 and IP2. In some tissues, such as parotid cells (Aub & Putney, 1984) Li+ results in an enhanced accumulation of polyphosphates. The effect of varying Li+ concentrations on the accumulation of inositol phosphates in hepatocytes indicates that Li+ inhibits the three enzymes, IP phosphatase and IP2 and IP3 phosphomonoesterase, with a half-maximal concentrations of 0.5, 1 and 5 mM respectively (Thomas et al., 1985). However, whether the kinetics of Li+ inhibition fit the model is unknown.

In summary, the data in the present investigations seem to support the existence of an IP3 generating mechanism and a degrading mechanism inside the Merkel cell-neurite complex. Specific steps during the two processes can be inhibited by neomycin and Li+ respectively. Although it is possible that PPI metabolism may play a role in transduction by Merkel cells, it is by no means the only mechanism involved. In the inner ear, for example, although various evidence suggests an importance of PPI metabolism in sound perception, a second messenger system would be too slow to account for the rapidity of the transduction process of vertebrate hair cells (Hudspeth,

1985). The characteristic slow time course of PPI hydrolysis in various tissues studied (Sekar and Hokin, 1986) would suggest a modulatory function of the PPI metabolism in Merkel cell-neurite complex.

In order to prove the existence of a second messenger, the putative messenger system should satisfy the following requirements: 1) specific synthesis of the messenger should occur during the stimulation and therefore its concentration should increase; 2)specific degrading mechanisms must be present to remove the messenger once the stimulation is over and 3)the putative second messenger must be able to mimic the effect of a natural stimulus. The first two points have been indirectly supported by the present experiments. However, in order to establish a second messenger role of IP3 the effect of IP3 administration into the cell should be investigated. Also, the level of IP3 inside the cell upon stimulation by its natural stimulation should be studied. At present no such experimental data are available. Experiments using in vitro preparations might be required to further study these effects.

A crucial event for the model will be the increase in intracellular concentration of Ca2+. In fact, as mentioned in Section 1.1.3, the importance of external Ca2+ has been confirmed and the release of intracellular Ca2+ from study on cells made permeable to Ca2+ is well

documented. Cytosolic Ca2+ may therefore be mobilized from either intracellular stores or from extracellular compartments or both. However the relative role of intracellular and extracellular Ca2+ is not known at present.

In conclusion, the present in vivo experiments indicate a possible existence of the phosphoinositide system similar to that suggested in other sense organs like hair cells of the acousticolateralis system. This may represent the process between the recognition of a mechanical stimulus and the Ca2+ signal which is responsible for secretory function of the Merkel cells. However, in order to prove a functional role of IP3, further experiments yielding more direct evidence must be carried out.

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